

SOIL SCIENCE

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NEW BRUNSWICK, N. J.



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PUBLISHED MONTHLY BY
WILLIAMS & WILKINS COMPANY
BALTIMORE, MD., U. S. A.

Entered as second-class matter May 12, 1919, at the post office at Baltimore, Maryland, under the act of March 3, 1879.

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Price per volume, net post paid { \$5.00, United States, Mexico, Cuba
\$5.25, Canada
\$5.50, other countries

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AQUEOUS VAPOR PRESSURE OF SOILS

MOYER D. THOMAS

Utah Agricultural Experiment Station

Received for publication January 31, 1921

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PART I

INTRODUCTORY

A knowledge of the soil solution *in situ* is highly desirable in soil studies. This applies to the mechanical action of the soil particles upon the solution and to the influence of any dissolved material such as soil alkali. When the soil solution is removed by centrifuging or by applying pressure, it is evident that the liquid so obtained cannot be expected to exhibit the same properties as when it is distributed over the surface of the soil particles. The vastly greater water-holding capacity of clay, as compared with sand, and also the undoubted absorption of such salts as sodium carbonate present two

problems which, with their manifold corollaries, demand for their solution a comprehensive method of attack. The experimental method described in this paper, as illustrated by a few preliminary results that are herein submitted, gives promise of throwing a flood of light upon both of these problems as well as upon many other problems arising out of them.

The most obvious physico-chemical method to use in studying the soil solution directly in the soil, is the freezing-point lowering. This has been extensively employed by Bouyoucos (8) and others. There are, however, several serious objections to this method. Since the soil cannot be thoroughly stirred during the process of freezing, the observed depressions do not indicate the true freezing points and the results are therefore at best merely relative. A more serious limitation lies in the fact that the method is not applicable to the drier soils. Again, it is possible that the relations which obtain at ordinary temperatures do not hold at the freezing temperature, though these discrepancies would probably not be of large magnitude.

The dilatometer method of Bouyoucos (6, 7), should theoretically be sufficient for the solution of the moisture-energy relations of the soil—at least over a wide range. The method seems to give very significant information over a range of moisture content from somewhat less than the wilting coefficient to very wet soils, but it has thus far failed to give any detailed information about the so-called "hygroscopic" moisture except perhaps to set its maximum amount and to indicate that it is held by very powerful forces. The experimental difficulties attending the general application of the method appear rather formidable.

The conductivity method (10) may serve a useful purpose in supplying qualitative evidence as to the soluble salt content of the soil solution when the moisture content is high, but this method also fails when applied to the drier soils, and moreover only relative and empirical results can be hoped for from it.

The methods which depend upon growing plants are likewise hampered by serious limitations. Fairly accurate results can be obtained for such approximate constants as the wilting point or the toxic limits of concentrations of alkali salts when large numbers of observations are made. However, since these constants vary within appreciable limits under different atmospheric conditions and under varying bacterial conditions, it is difficult to assign any very exact theoretical significance to them.

The vapor pressure of the soil solution should supply an abundance of quantitative information about the soil. For this function, being a property of the free energy at the surface of the moisture film, is fundamentally bound up with such other properties as the osmotic pressure, freezing-point, surface tension, capillary potential, moisture equivalent, concentration of dissolved substances, and the effective diameter of the soil particle (that is, the total surface of the soil). Thermodynamic relations are known, or can be deduced, by which most of these can be calculated directly from the vapor pressure.

HISTORICAL

Highly precise measurements of the vapor pressures of certain solutions have been made by several investigators. Two general methods have been used: (1) the static and (2) the dynamic or air-saturation method.

The static method, in which the pressure differences are observed directly on manometers, was never applied to solutions with notable success until Frazer and Lovelace (15, 16, 17, 18) made it a process of great precision. Preliminary experiments in this laboratory indicated that the experimental difficulties of applying it to soils would be even greater than to solutions, and so it was temporarily abandoned in favor of the dynamic method. It is hoped, however, that the work on the static process can be resumed in the near future.

The dynamic method consists essentially in passing a known volume of air through a substance, collecting and weighing the vapor, and computing the partial pressure from the gas laws. It was first used by Regnault (29) in 1845, and it has gone through many transformations of procedure and application in the hands of Tammann (30), Walker (31), Will and Bredig (33), Orndorff and Carrell (26), Perman (28), Carveth and Fowler (13), Kahlenberg (22), Berkeley and Hartley (4, 5), Lincoln and Klein (24), Krauskopf (23), Derby, Daniels, and Gutsche (14), Washburn and Heuse (32), Baxter and Lansing (2), and others. So far as is known the dynamic method has not yet been applied to soil studies.

The greatest success in applying the method to solutions seems to have come from a suggestion of Ostwald, which was first carried out by Walker (31), that the process should be made differential by passing the same air first through the solution and then through the pure solvent, thus obtaining the relative vapor pressures of the two liquids. The apparatus was greatly improved by Kahlenberg (22) and by Berkeley and Hartley (5) who constructed saturators consisting of a series of horizontal parallel tubes connected at the ends by inverted U-tubes. The gas was passed over the surface of the liquid in these vessels while they were being rocked gently, thus causing the liquid to flow back and forth from one end to the other. Dry air was sent into the first saturator containing the solution and from there passed into a second saturator containing water. From the losses of weight of the two vessels, the vapor-pressure lowering of the solution could be readily deduced without a knowledge of the air volume. The results were very exact.

Washburn (32) made a slightly different application of the differential method. He used saturators of the same form as the above, but they were much larger and thus permitted a faster air current. Instead of weighing the saturators he absorbed the moisture from each in small absorbers containing sulfuric acid and phosphorus pentoxid. Excellent results also were obtained by this process.

A few vapor-pressure measurements of soils are recorded by Patten and Gallagher (27) who let the soil stand at 25°C. over sulfuric-acid solutions of known strength and vapor pressure, and observed the equilibrium moisture content which the soil approached from both a drier and a wetter condition.

EXPERIMENTAL

Description of apparatus

The method employed in the present experimental work is a combination of the methods of Berkeley and Washburn with some minor modifications. The apparatus is shown diagrammatically in figure 1. Its essential features consist of the following:

(1) *The constant level reservoir "A,"* from which water is siphoned under uniform head into the bottles "C" or "D" in order to force air through the apparatus.

(2) *The feed device "B,"* which serves to prevent air from collecting in the feed tubes and thus altering the rate of flow of the water.

(3) *The bottles "D" and "D₁,"* whose volumes are accurately known. The volume of the air used in an experiment can be accurately determined by adding 1 or 2 liters of water at room temperature, closing the bottle with a stopper and thermometer, and shaking until constant temperature is attained. The air is then practically saturated with water vapor at known temperature and pressure. The bottle is quickly placed in position. Knowing the time-weighted barometric pressure during the run, it is easy to calculate the total volume that passes through the apparatus. A check is thus obtained on the process by calculating the vapor pressure of water at 25°C.

(4) *The trap bottle "E."*

(5) *The loosely packed soda-lime tube "F,"* which removes any carbon dioxide.

(6) *The flowmeter "G" (3),* which consists of an oil manometer for measuring the pressure difference on the two sides of a capillary tube through which the gas is forced. This instrument must be calibrated empirically with known rates of speed, and from the curve so obtained the speed of the gas can be read directly.

(7) *The bulbs "H,"* which contain a little water over which the gas passes and becomes nearly saturated with water vapor before entering the soil tube "K."

(8) *The brass soil cylinders "K" and "K₁,"* which are 2 by 10 inches in dimensions and are connected in series by glass tubing so that the air can enter at the bottom of each one in turn. They are filled with the soil and closed by means of paraffined rubber stoppers. An oil manometer "M₂" standing in the rubber stopper attached to the second tube indicates the pressure at this point.

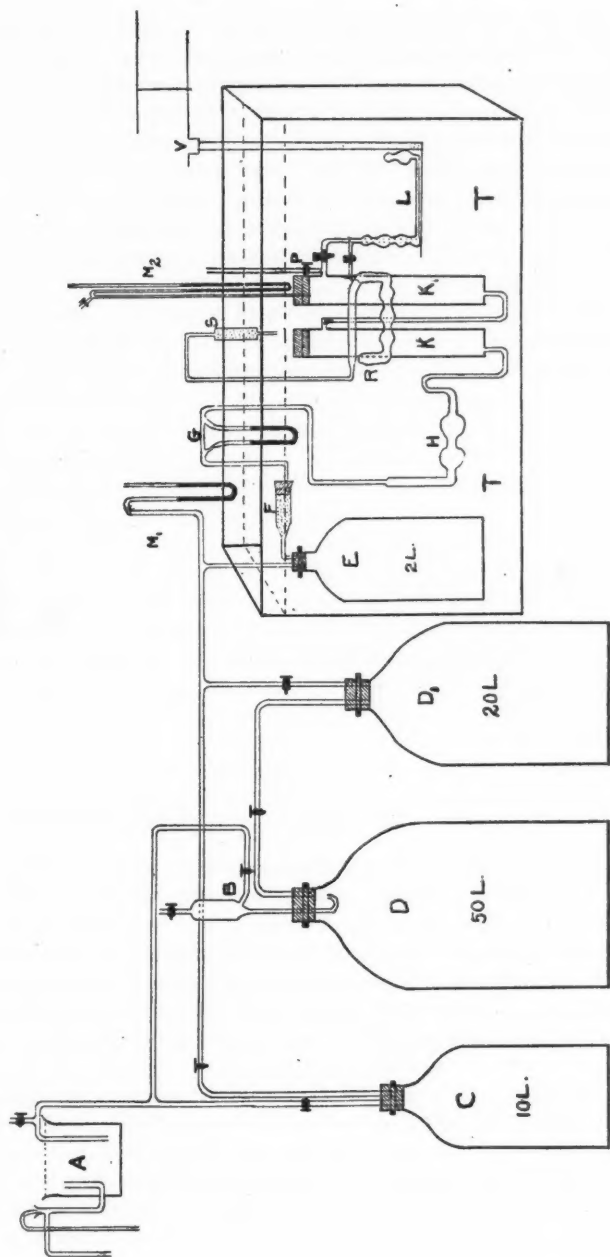


FIG. 1. DIAGRAM OF APPARATUS FOR MEASURING THE VAPOR PRESSURE OF SOILS
(See description in text)

(9) *The saturator "L."* This vessel is illustrated in figure 2. Its intake tube is fastened to the outlet of "K." It is also tied to a wire basket, the arm of which engages a rotating lever at "V" causing the outer end of "L" to be raised and lowered about four times a second. The tubes of the saturator have an internal diameter of 12 to 14 mm., and just enough water is placed in them so that the air is not at any time forced to bubble through it. The vessel is provided with two well seated glass taps. When these are lubricated with a heavy rubber grease they are unaffected by immersing in water, but it is expedient to cut off the prongs of the taps and to put two rubber caps on each of them during the run.

(10) *A glass absorber "R,"* which is illustrated in figure 3. This vessel is about 7 inches long and the two lower tubes contain about 40 gm. of sulfuric acid. The upper tube contains phosphoric anhydride on glass wool. Ten

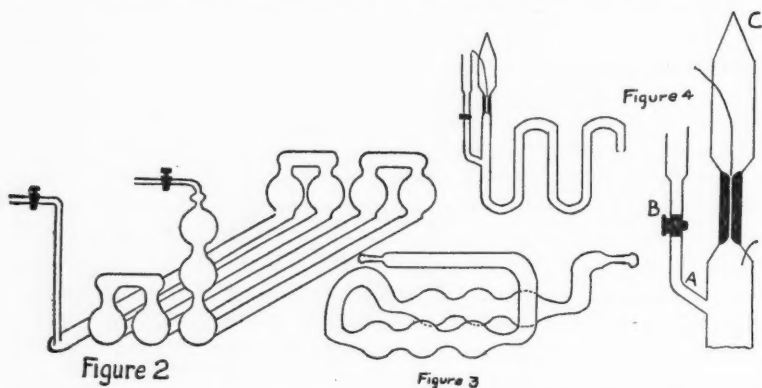


FIG. 2. THE SATURATOR

FIG. 3. THE ABSORBER

FIG. 4. THE THERMOREGULATOR

grams of water can be absorbed with this arrangement without wetting the phosphoric anhydride appreciably. The acid should perhaps be changed when 6 to 8 gm. have been absorbed. The absorber is connected to the exit tube of "L" and placed in a horizontal position. The motion of "L" serves to keep the surface of the acid agitated, thus bringing about efficient absorption. Well ground glass caps are provided for this vessel while it is being weighed.

(11) *The calcium chloride tube "S,"* which prevents back diffusion of water vapor.

(12) *The thermostat "T."* Careful temperature control is an absolute necessity in view of the fact that the temperature coefficient of the vapor pressure of water at 25°C. is about 1.3 mm. per degree, or three times the depression caused by dissolving a mole of glucose in 1,000 gm. of water. This differential method should not, however, be influenced by a slow fluctuation of temperature, provided the bath is well stirred, because the ratio of the

vapor pressure of a solution and its solvent remains practically constant over a considerable range of temperature. But in this apparatus the heat capacities of the soil cylinders and the saturator are so different that a fluctuation of temperature is disastrous.

The thermostat consists of a 30-gallon tank heated by means of electric-light globes and well stirred by a $\frac{1}{8}$ -h.p. motor. The thermo-regulator (fig. 4) is the usual mercury-toluene type, made of a thin-walled glass tube 10 mm. in diameter and about 10 feet long, bent to fit along the back of the tank. It might be well to point out one of the features of this regulator, devised by the writer, which makes it almost perfect in its operation.

The mercury end of the regulator with its two sealed-in platinum connections is shown in figure 4. The space above the capillary tube is filled with hydrogen and sealed. With this arrangement no fouling of the mercury contact has been observed after over a year of service. In filling the regulator care must be exercised to get rid of all toluene from the mercury end. This is accomplished by adding mercury to within a few centimeters below the side arm "A" (fig. 4). With the drawn-out end "C" open, air is sucked through the capillary tube until the toluene is removed. This space is partially exhausted while the tube below is heated to remove the toluene adhering to the glass under the mercury. The regulator is now filled with mercury, and hydrogen is introduced into the space above the capillary by raising and lowering the mercury, while a stream of gas is sent through a T-tube connected at "C." When the compartment is full of hydrogen the tube "C" is quickly sealed off. It is best to leave the gas under slight pressure so that when the tap "B" is opened the mercury will fall just below the top of the capillary. It is then very easy to set the instrument for operation. No variation in the temperature of the thermostat has been observed on a sensitive Beckman thermometer over long periods of time when the regulator was immersed so that the tap "B" and the platinum connection "E" were just above the surface of the water.

Preparation of the soil

Though it is quite possible that the method of preparing the soil may be a factor which influences the vapor pressure, the following process is the only one that has thus far been used in this investigation.

A large sample of air-dry soil is put through a 1-mm. sieve and thoroughly mixed. For each preparation, about a kilo of soil is mixed with the requisite amount of water or salt solution and the whole mass is forced through the same sieve twice. The material is then closed up in a 2-quart fruit jar and shaken vigorously at intervals for one or two days before use. Those soils which are wet enough to form a solid lump are shredded by forcing them through a $\frac{3}{16}$ -inch sieve. The material is placed in the brass soil tubes ("K," fig. 1) in as loose a condition as possible at the time of commencing the experi-

ment. Care should be taken to smooth the surface of the soil in the second tube, but obstructions of cotton or glass wool must not be placed in the tube leading to the saturator. Menzies (25) has shown that glass wool filters can remove appreciable amounts of water vapor from the air. With the speed of air employed in this work no trouble has been experienced in having solid particles carried over.

Weighing the saturator

The most important and the most difficult single operation in the experiment is the weighing of the saturators. An error of 1 mgm. in the weight of the saturator means an error of 0.01 mm. in the vapor pressure of the soil at 25°C. when 100 liters of air are passed. The weighing errors have been reduced to about 0.2 mgm. or less by the following method:

A balance room, maintained nearly at constant temperature and free from dust and jars, has been prepared in the center of the building. A high-class balance provided with cup-shaped pan supports is used and the balance is kept in a dust-proof box when not in use. The internal and external volumes of the saturators, and also of a sealed-up counterpoise of about the same size and shape, are known. The saturators are closed before removing from the thermostat, the internal pressure being noted, and they are not opened again during the weighing. The vessels are left in the balance for several hours before weighing them. The weights are then corrected to an average internal pressure and average balance conditions. Great accuracy is not so necessary in weighing the absorbers, but these have always been properly counterpoised and weighings corrected.

Conducting the experiment

The technique of the experiment is very simple. The saturator and the absorber are first weighed. Then the taps of the saturator are enclosed with rubber caps and the vessel is fastened to its basket. The soil cylinders are filled and connected to the saturator and absorber with rubber tubing, all rubber connections being securely wired with fine copper wire. The apparatus is put into position in the thermostat where it remains for nearly an hour in order to take the bath temperature before commencing the run. During this time the bottles "D" and "D₁" are prepared and a slow current of air from the bottle "C" is sent through the soil tubes and out through the clamp "P." This clamp is then closed, and the pressure in the apparatus is adjusted to the value which previous experiments have indicated to be necessary to send the gas through the phosphoric anhydride of the absorber (from 5 to 40 mm. of oil). The bottle "C" is now cut off and the bottles "D" and "D₁" are put into communication with the system. The pressure is again adjusted before the saturator stopcocks are opened to commence the experiment. A speed of 2 liters per hour has finally been adopted after many preliminary

experiments had indicated that erratic results are obtained when the speed is 3.5 liters per hour or greater. From 50 to 150 liters of gas are passed in an experiment. The large bottles enable the run to proceed night and day without attention, except to prevent large and sudden fluctuations in the temperature of the room. In actual practice two complete and independent sets of apparatus have been constructed so that two experiments can go on simultaneously. Enough extra saturators and absorbers also are at hand to avoid loss of time in starting a new run.

The moisture determinations have been carried out by heating 5 to 15-gm. samples of the soil to 110° to 112°C. to constant weight in a large oven into which a current of dry air was sent intermittently. The drying usually extended over several days, and even then satisfactory results were not always obtained. Indeed, an error of at least 0.05 per cent in the moisture content of the soils is probably present in most cases, due perhaps to errors in sampling and drying. This error becomes quite significant in the drier soils. Moisture determinations also were made on the soil at the top of the second tube at the end of the experiment, but no significant changes were detected. It is proposed to try to find a more accurate method of determining the moisture before continuing the work.

The soils

Four soils have been studied: (a) sand from River Heights, Logan; (b) Greenville silty clay loam; (c) clay loam from Sixth West Street, Logan; (d) a silty clay separate, the tenth soil separate prepared by Gardner (19) in his elutriator.

The mechanical analyses of these soils are given in table 1.

TABLE 1
Mechanical analyses and surface areas of the soils

SIZE			SAND		GREENVILLE LOAM		WEST LOGAN CLAY LOAM		CLAY SEPARATE	
Limits		Mean	Per cent	Area*	Per cent	Area	Per cent	Area	Per cent	Area
<i>mm.</i>		<i>mm.</i>								
1.0 -0.5		0.75	6.96	2	0.34		0.22			
0.5 -0.25		0.37	8.91	2	0.41		0.51			
0.25 -0.1		0.17	57.68	74	4.60	6	4.87	6		
0.1 -0.05		0.075	18.46	54	19.81	57	21.98	65		
0.05 -0.01		0.025	3.00	26	40.06	345	40.44	359		
0.02 -0.01		0.015							46.8	673
0.01 -0.005		0.0075	0.80	23	10.11	319	4.88	145	16.7	480
0.005 -0.001		0.0025	1.67	146	12.85	1,105	10.67	946	21.4	1,847
0.001 -0.0		0.0005	3.21	1,405	14.44	6,220	17.43	7,740		
0.001 -0.0005		0.00075							12.9	3,710
0.0005 -0.0		0.00025							10.5	9,060
Total			100.69	1,732	102.62	8,052	101.00	9,261	108.3	15,770

* Square centimeter per gram.

Other physical properties are given in table 2.

TABLE 2
Some physical properties of the soils under investigation

PROPERTY	SAND	GREENVILLE LOAM	WEST LOGAN CLAY LOAM	CLAY SEPARATE
Moisture-holding capacity (Hilgard) (per cent).....	27.90	45.80	47.60	82.00
Moisture equivalent (per cent).....	3.00	23.30	24.20	35.70
Wilting coefficient (Briggs and Shantz) (per cent).....	1.39	9.30	11.00	15.00 (calc.)
Air-dry moisture content (per cent).....	0.30	2.64	2.69	4.50
Real specific gravity 25°	2.750	2.791	2.695	2.783

Complete chemical analyses of these soils have not been carried out, but their water-soluble, and carbonate contents and their organic-carbon contents as determined by the wet combustion method are given in table 3.

TABLE 3
Partial chemical analyses of the soils

	TOTAL SOLUBLE	CARBONATE (CO ₂)	ORGANIC CARBON (C)
	per cent	per cent	per cent
Sand.....	0.0382	18.9	0.323
Greenville loam.....	0.1060	18.7	1.320
West Logan clay loam.....	0.1300	14.4	1.030
Clay separate.....	0.0573	15.1	0.125

In the case of the Greenville soil a few vapor-pressure determinations have been made with some soluble salts added, and in table 4 are given analyses of these soils. The water extraction was made by shaking 50 gm. of soil with 500 cc. of water for 10 minutes and filtering through a Pasteur-Chamberland filter. Only the negative ions were determined in the extracts and the results were expressed as sodium salts.

TABLE 4
Salts extracted from Greenville clay loam with various salts added

SALTS ADDED	NaCl	Na ₂ SO ₄	Na ₂ CO ₃	ADDED SALTS EXTRACTED
	per cent	per cent	per cent	per cent
None.....	0.0120	0.0196	0.021	
0.300 per cent NaCl.....	0.310			99.4
0.729 per cent Na ₂ SO ₄		0.750		100.2
0.544 per cent Na ₂ CO ₃			0.210	36.6
1.088 per cent Na ₂ CO ₃			0.450	39.4

Results

The vapor-pressure results thus far obtained are recorded in table 5 and are plotted graphically against the moisture contents in figure 5. In their form these curves are approximately rectangular hyperbolae, as figure 5 indicates.

TABLE 5
Vapor pressure data

SOIL	SALT ADDED	DATE (1920)	SPEED OF AIR PER HOUR	WATER IN SOIL	VAPOR PRES- SURE, WATER	VAPOR PRESSURE SOIL	
						Mercury column	Mean
	<i>per cent</i>		<i>liters</i>	<i>per cent</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
Sand.....	None	November 27	2.0	0.70	22.90*	22.737	22.737
	None	November 26	2.0	0.90	23.40*	23.332	23.332
	None	November 27	2.0	1.14	23.00*	23.491	23.491
	None	November 24	2.0	1.63	23.50*	23.579	23.579
	None	December 27	2.0	4.20		23.714	23.714 (?)
Greenville clay loam..	None	July 5	3.0	2.65		10.60	
	None	July 6	3.0	2.65		10.710	10.65
	None	October 22	2.0	4.45	23.80	20.628	
	None	October 24	2.0	4.45	23.80	20.537	20.582
	None	November 10	2.0	5.10	23.90	21.727	21.727
	None	August 21	2.7	6.44	23.78	22.950	
	None	August 25	2.7	6.44	23.92	22.967	22.958
	None	October 18	2.0	8.31	23.88	23.405	
	None	October 21	2.0	8.31	23.90	23.370	23.387
	None	November 10	2.0	9.52	23.88	23.470	23.470
	None	October 23	2.0	17.25	23.85	23.712	
	None	October 25	2.0	17.25		23.725	23.719
Greenville clay loam..	0.3 NaCl	July 31	4.5	4.88	23.83	20.666	
	0.3 NaCl	August 4	3.0	4.88	23.90	20.780	20.723
	0.3 NaCl	July 21	3.0	5.71	23.90	21.888	
	0.3 NaCl	July 26	4.0	5.71	23.85	21.818	21.853
	0.3 NaCl	July 29	3.0	7.12	23.80	22.890	22.890
	0.3 NaCl	December 26	2.0	7.00	23.76	22.855	22.855
	0.3 NaCl	August 5	3.0	8.87	23.76	23.165	
	0.3 NaCl	August 7	3.3	8.87	23.70	23.121	23.143
	0.3 NaCl	August 10	4.4	17.00	23.85	23.576	
	0.3 NaCl	August 12	3.9	17.00	23.90	23.506	23.529
	0.3 NaCl	August 16	3.2	17.00	23.90	23.506	
	0.3 NaCl	August 11	2.8	24.90	23.85	23.670	
	0.3 NaCl	August 13	3.0	24.90	23.85	23.570	23.603
	0.3 NaCl	August 16	3.8	24.90	23.92	23.568	

TABLE 5—Continued

SOIL	SALT ADDED	DATE (1920)	SPEED OF AIR PER HOUR	WATER IN SOIL	VAPOR PRESS- URE, WATER	VAPOR PRESSURE SOIL	
						Mercury column	Mean
	<i>per cent</i>		<i>liters</i>	<i>per cent</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
Greenville clay loam.	0.544 Na ₂ CO ₃	August 17	2.7	5.35	23.90	21.961	
	0.544 Na ₂ CO ₃	August 19	2.7	5.35	23.87	21.988	21.974
	0.544 Na ₂ CO ₃	August 18	3.0	6.33	23.84	22.913	
	0.544 Na ₂ CO ₃	August 21	2.9	6.33	23.90	22.915	22.914
	0.544 Na ₂ CO ₃	August 23	3.4	7.96	23.90	23.198	
	0.544 Na ₂ CO ₃	August 24	3.0	7.96	23.95	23.278	23.238
	0.544 Na ₂ CO ₃	August 26	2.9	9.80	24.05	23.487	
	0.544 Na ₂ CO ₃	August 28	3.1	9.80	23.90	23.465	23.476
	0.544 Na ₂ CO ₃	August 31	3.2	15.05	23.85	23.650	
	0.544 Na ₂ CO ₃	September 3	3.1	15.05	23.90	23.661	23.656
	0.544 Na ₂ CO ₃	October 12	2.3	22.50	23.80	23.713	
	0.544 Na ₂ CO ₃	October 15	2.2	22.50	23.83	23.739	23.726
Greenville clay loam	1.088 Na ₂ CO ₃	November 2	2.0	6.77	23.90	23.009	
	1.088 Na ₂ CO ₃	November 5	2.0	6.77	23.87	22.962	22.986
	1.088 Na ₂ CO ₃	November 20	2.0	11.25	23.90	23.448	
	1.088 Na ₂ CO ₃	November 22	2.0	11.25	23.50*	23.481	23.464
Greenville clay loam.	0.729 Na ₂ SO ₄	November 18	2.0	7.39	23.90	23.067	
	0.729 Na ₂ SO ₄	November 18	2.0	7.39	23.95	23.044	23.056
	0.729 Na ₂ SO ₄	November 22	2.0	10.15	23.80	23.407	23.407
West Logan clay loam.	None	November 29	2.0	2.69		11.700	11.700
	None	December 1	2.0	5.60	23.90	22.528	22.528
	None	December 3	2.0	7.35	23.88	23.168	23.168
	None	November 29	2.0	9.55	23.85	23.490	23.490
	None	December 16	2.0	22.30		23.709	23.709 (?)
Clay separate	None	December 5	2.0	5.59		16.100	16.100
	None	December 10	2.0	8.27	23.90	22.058	22.058
	None	December 6	2.0	10.52		22.910	22.910
	None	December 20	2.0	11.30	23.85	23.011	23.011
	None	December 8	2.0	15.70	23.80	23.525	23.525
	None	December 7	2.0	29.60	24.00	23.718	23.718
	None	December 9	2.0	29.60	23.80	23.719	23.719

* A slight leak in preliminary gas train.

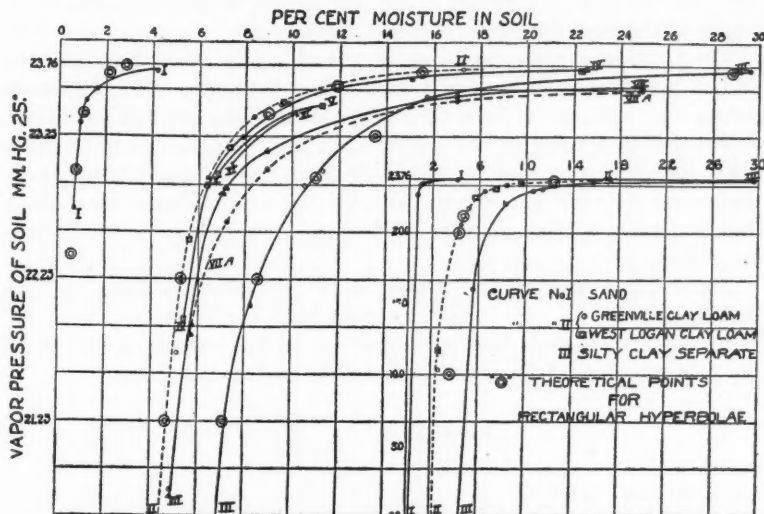


FIG. 5. DIAGRAM SHOWING VAPOR PRESSURE IN MILLIMETERS OF MERCURY AT 25°C. OF FOUR DIFFERENT SOILS CONTAINING VARIOUS QUANTITIES OF MOISTURE

Curve I sand, no salt added; Curve II Greenville clay loam and West Logan clay loam, no salt added; Curve III silty clay separate, no salt added; Curve IV Greenville clay loam plus 0.544 per cent Na_2CO_3 ; Curve V Greenville clay loam plus 1.088 per cent Na_2CO_3 ; Curve VI Greenville clay loam plus 0.729 per cent Na_2SO_4 ; Curve VII Greenville clay loam plus 0.3 per cent NaCl ; Curve VII-A, theoretical curve for Greenville clay loam plus 0.3 per cent NaCl .

The vapor pressure is thus a linear function of the reciprocal of the moisture content over a wide range. The equations representing the curves are:

$$\text{Sand: } (23.90 - p)(p - 0.35) = C_1 = 0.36$$

$$\text{Greenville loam and West Logan clay loam: } (23.96 - p)(p - 3.5) = C_2 = 3.1$$

$$\text{Clay separate: } (23.96 - p)(p - 4.8) = C_3 = 6.2$$

where p is the vapor pressure of the soil and p is the per cent of moisture. The significance of the constant C is of interest. It is the square of distance from the asymptotes to the point of maximum curvature and may therefore become a very important constant for characterizing the texture of the soil. The upper asymptote is very close to 23.96 in the case of the heavier soils and 23.90 in the case of the sand. This simply means that the moisture surfaces should proceed gradually from negative curvature, through the flat surface condition to positive curvature as the moisture content increases. It is doubtful if this positive curvature could be realized in practice. The curves actually cross the other asymptote in the dry soils, and according to the observations of Patten and Gallagher (27) bend off toward the origin. It is probable that the origin is not reached at zero vapor pressure, and accord-

ingly the curves have been tentatively produced to the zero ordinate. This region will be made the subject of special study later.

The positions of the curves seem to depend largely on the texture of the soil, but other factors no doubt exert minor influences. Thus the West Logan clay loam, which from the mechanical analysis, moisture equivalent, moisture-holding capacity, and wilting point must be presumed to be somewhat finer in texture than the Greenville loam, has a vapor pressure curve almost exactly coincident with the latter's. It is possible that the somewhat higher organic carbon content of the Greenville soil accounts for this coincidence.

The observed vapor pressure of water at 25°C. is included whenever possible as a check on the run. As no great effort was made in most cases to determine the exact time-weighted barometric pressure, slight variations in this value are to be expected. It was calculated from the gas laws as follows:

$$pV = NRT = \frac{WRT}{18.016}$$

where W is the weight of water absorbed, V the total volume of gas in liters that passed through the saturator and is equal to the volume of the air plus the water vapor, T the absolute temperature (298°), R the gas constant (62.4 liter-mm. of mercury), and p the partial pressure in millimeters of mercury. The formula becomes

$$p = \frac{62.4}{18.016} \frac{WT}{V} = 3.4613 \frac{WT}{V}$$

The best observed values for the vapor pressure of water at 25°C. seem to approximate 23.85 mm. As the thermostats were set at 25.08° on a thermometer graduated to tenths which gave an ice point of 0.16° and the melting point of sodium sulfate decahydrate as 32.38° (true melting point 32.384°), it is not impossible that the thermometer was correct at 25°. In that case the vapor pressure at 25° would be nearly 23.75 mm., and this value is very close to the probable value of 23.76 mm., as found by other investigators (12, 22).

Calculating vapor pressure of the soil

The vapor pressure of the soil was deduced as follows:
In the equations:

$$pV = W \frac{RT}{18.016} \quad (1)$$

$$p_o V_o = W_o \frac{RT}{18.016} \quad (2)$$

let p be the partial aqueous vapor pressure, V the total volume of the gas passing through the soil, W the weight of water in the gas in equilibrium with the soil, and p_o , V_o , and W_o the corresponding values for the same gas in the saturator.

Then dividing (1) by (2) and solving for p we get:

$$p = \frac{W}{W_o} \cdot \frac{V_o}{V} \cdot p_o$$

It is obvious that if the gas absorbs additional water vapor in the saturator V_o will be greater than V . In any case these volumes will be inversely proportional to the partial pressure of the air in them and we can write:

$$\frac{V_o}{V} = \frac{B - p}{B - p_o}$$

where B is the barometric pressure. Therefore,

$$p = \frac{W}{W_o} p_o \frac{B - p}{B - p_o}$$

Solving,

$$p = \frac{W p_o B}{B W_o + p_o (W_o - W)}$$

The value of p_o was taken as 23.760.

Good concordance was generally obtained in duplicate determinations, and it is believed that most of the later values reported are within 0.01 to 0.02 mm. of the correct values. In the case of the drier soils greater divergences are likely, but great exactness in these regions has no real significance because of errors in moisture determinations. It should be emphasized that the results in this paper are of preliminary nature only and that they are reported to illustrate the possibilities of the method rather than to give highly exact measurements. Slight changes in the apparatus were being continually made during the progress of this work, and therefore the later experiments are probably a little more accurate than the earlier ones. In its final form the apparatus gives consistent results to 0.01 mm. Still greater accuracy will very likely be possible when a contemplated improvement is embodied in the apparatus by which the slight resistance of the soil column to the air current will be reduced practically to zero.

PART II

DISCUSSION OF SOME POSSIBLE APPLICATIONS OF VAPOR PRESSURE DATA

The vapor pressure of the soil solution is fundamentally a result of the configuration and composition of the liquid phase, and two general factors may be recognized by which this function is characterized: (a) the curvature of the water wedges between the soil particles due to the surface tension of the liquid, and (b) the attractive forces of the soil mass acting upon the surface of the moisture film due to the thinness of the film. The magnitude of each of these forces will be a function of the moisture content. The influence of dissolved substances might be supposed to be a third independent variable, but since this factor can be calculated from a knowledge of the laws of solutions (if the soil is characterized for zero or a known concentration) it is obviously implicitly involved in the first two factors.

Surface tension, curvature, and vapor pressure

The relation between curvature, surface tension, and vapor pressure lowering can readily be found from a speculation involving a capillary tube of radius r standing in a liquid of surface tension t at a constant temperature T . At equilibrium the vapor pressure p at the top of the tube will be equal to the vapor pressure p_o of the flat surface below diminished by the weight of the vapor column of height h . This relation is expressed exactly by the following equation, if the vapor obeys the gas laws:

$$h = \frac{RT}{Mg} \log_e \frac{p_o}{p} \quad (1)$$

in which M is the molecular weight of the liquid and g the acceleration of gravity.

The corresponding relation between the surface tension and hydrostatic pressure is

$$h = \left(\frac{2t}{Mg} \cdot \frac{V_o}{r} \right) + (p_o - p) \frac{V_o}{Mg} \quad (2)$$

where V_o is the molar volume of the liquid.

Combining equations (1) and (2) we get:

$$\log_e \frac{p_o}{p} = \left(\frac{2t}{r} \cdot \frac{V_o}{RT} \right) + (p_o - p) \frac{V_o}{RT} \quad (3)$$

This equation states as r approaches ∞ , p approaches p_o , and as r approaches zero, p also approaches zero. Accordingly, the vapor-pressure-moisture curves should pass through the origin and perhaps be asymptotic to the

ordinate representing the vapor pressure of water. The intermediate portions of the curves will depend on the rate of change of r with the moisture content, which in turn will depend on the size of the soil particles.

The fact that the curves do not pass through the origin, but rather seem to strike the zero ordinate at appreciable moisture contents, indicates probably that as the soil becomes drier the negative curvature of the water wedges spoken of by Briggs (9, p. 19), is being augmented by the attractive forces of the soil particles acting through the thin water film to its surface. At some point on the curve the water wedges may disappear entirely and we shall be concerned only with very thin films of moisture covering the surfaces. These films may eventually become so thin that they do not exert any appreciable vapor pressure.

Capillary potential and vapor pressure

The capillary potential function " ψ " (12) is the work that must be done by the field forces in removing one mole of water from the body of the soil solution to infinity. A simple derivation involving the condensation or evaporation of 1 gm. of the vapor whose molar volume is v_o under its own pressure p_o in a large cylinder gives the relation:

$$-\psi = \frac{L_o - p_o (v_o - V_o)}{V_o} = \frac{L_o - RT}{V_o} \quad (4)$$

where L_o is the ordinary molar heat of evaporation.

If this process is now carried out at the same temperature in a capillary tube of radius r , the vapor pressure of the liquid will be lowered to p , and in order to cause evaporation from the curved surface it will be necessary to supply an amount of heat, in addition to L_o , equal to the Laplacian capillary pressure P , since the density of water is unity. The relation between them becomes:

$$-\psi = \frac{L_o - RT}{V_o} + P \quad (5)$$

but by definition

$$P = \frac{2t}{r} \quad (6)$$

and from equation (3) above

$$\frac{2t}{r} = \frac{RT}{V_o} \log_e \frac{p_o}{p} - (p_o - p) \quad (7)$$

Therefore,

$$-\psi = \frac{L_o - RT}{V_o} - \frac{RT}{V_o} \log_e \frac{p}{p_o} - (p_o - p) \quad (8)$$

The term $\log_e \frac{p}{p_o} = \log_e \left(\frac{p - p_o}{p_o} + 1 \right)$

can be expanded into a power series, and for values of p fairly close to p_o , only the first term is significant, and we can write, neglecting also the term $(p_o - p)$:

$$-\psi = \frac{L_o - RT}{V_o} - \frac{RT}{V_o} \cdot \frac{p}{p_o} + \frac{RT}{V_o} \quad (9)$$

If we collect the constant terms and notice that from figure 5 the vapor-pressure-moisture curves are approximately rectangular hyperbolae and therefore

$$(a - p) = \frac{c}{(\rho - b)}$$

where ρ is the moisture content, we get:

$$-\psi = A + B \frac{c}{(\rho - b)} = A + \frac{D}{(\rho - b)} \quad (10)$$

where a, b, c, A, B , and D are constants.

This is the form, at least, of Gardner's potential-moisture function as deduced from Briggs' data (20, 21). It may be expected to hold over the range of moisture contents within which water can move through the soil by capillarity, for both the approximating assumptions that were made are quite valid under these conditions.

Vapor pressure and total surface of the soil

The exact experimental measurement of the total surface of a soil would be very difficult by a mechanical analysis on account of the fact that particles of lower diameter than 0.001 mm. generally contribute the largest proportion of the total surface. The equation,

$$\text{Total surface} = \frac{6}{D \times \text{sp. gr.}} \text{ sq. cm. per gram}$$

where D is the "effective" diameter of the soil in centimeters, indicates that the surface is inversely proportional to D . This might mean, to take an extreme example, that a sand containing 1 per cent of colloid of diameter 0.00001 mm. would have about the same total surface as a clay of a mean diameter of 0.001 mm.

One might reasonably expect, however, that the ratios of the moisture contents at zero vapor pressure, or indeed on any of the equi-vapor-pressure ordinates, along the nearly vertical parts of the curves, would approximate closely the ratios of the total surfaces of the soils. At higher moisture contents the smaller particles would very likely be completely immersed in the liquid so that the apparent total surface would be changed.

The lack of good concordance between the ratios of the moisture contents at about 10 mm. pressure, and the ratios of the calculated total surfaces is probably due, at least in part, to the insufficiency of the data given by the mechanical analysis for calculating the total surface (table 1).

Hygroscopic coefficient and vapor pressure

The explanation of the great value of the hygroscopic coefficient for characterizing soils follows at once from the above considerations. This moisture content, which represents an equilibrium condition between the vapor pressure of the soil and the partial pressure of the water vapor in the air of a room, is generally rather low down on the vapor pressure curves. It will be observed from the curves that with relative humidities from zero up to 90 per cent the range of moisture content is only from 1 to 4 per cent, according to the texture. Therefore, if care is taken to control the relative humidity, to say 10 per cent, the moisture content would be accurate to 0.1 to 0.4 per cent. It should be emphasized, however, that the term "hygroscopic coefficient" has no real meaning unless the relative humidity is specified. For if a soil were allowed to stand long enough in a completely saturated atmosphere it would go on taking up moisture until the water wedges attained zero curvature. This would probably result in a very wet soil. The experiments of Patten and Gallagher (27) seem to bear out this conclusion, though they emphasize the fact that equilibrium is attained very slowly. More rigid temperature control than 0.1° is necessary to give the experiments finality. It is not unlikely, however, that every soil is characterized by more than one vapor pressure-moisture curve according to the mode of preparation, in which case the above conclusion would have only limited application. Experiments are now being planned to investigate this matter.

The wilting coefficient and vapor pressure

The values of this soil constant, as determined by the method of Briggs and Shantz (6) are given in table 2 for three of the four soils. Tepary beans were grown. The numbers given are the averages of 75 tumbler determinations. The wilting point for wheat also was determined on Greenville soil. The clay separate was not investigated, but its wilting coefficient has been calculated from the moisture equivalent by dividing by 2.4, which was the ratio for the other soils. It will be seen from table 2 and figure 5 that the

plants invariably wilted when the vapor pressure of the soil commenced to fall rapidly. The vapor-pressure depressions corresponding to these moisture contents were as follows:

(1) Sand (beans).....	$W = 1.39$ per cent H_2O	$(p_o - p) = 0.20$ mm.
(2) Greenville loam (beans).....	$W = 9.30$ per cent H_2O	$(p_o - p) = 0.30$ mm.
(3) Greenville loam (wheat).....	$W = 7.70$ per cent H_2O	$(p_o - p) = 0.46$ mm.
(4) West Logan clay loam (bean).....	$W = 11.00$ per cent H_2O	$(p_o - p) = 0.20$ mm.
(5) Clay separate.....	$W = 15.00$ per cent H_2O (calc.)	$(p_o - p) = 0.30$ mm.

It is evident from the above discussion that the wilting point for each soil can be characterized fairly definitely from its vapor-pressure curve. Naturally, in the direct determination of this value the atmospheric temperature and humidity will play important rôles and should be controlled. A very serious error results from the difficulty of judging when the plants are wilted. The inconsistencies in the literature in the wilting-point determinations are no doubt assignable to these causes. The function might be more accurately defined in terms of a definite vapor-pressure depression. This would mean that plant roots would have to exert a definite arbitrary force at this point to take up the water from the soil, whether the plant leaves were transpiring the moisture at the same rate as the intake at the roots or not. It might be mentioned that a vapor-pressure depression of 0.4 mm. in a solution at 25° means an osmotic pressure of about 26 atmospheres.

"Unfree" water and vapor pressure

In determining the "unfree" water in a soil Bouyoucos (6) measures the amount of water that does not freeze at -4° . When equilibrium is reached the moisture remaining on the soil will have the same vapor pressure as the ice at -4° . Assuming von Babo's law to hold, one finds from the data of Frazer, Lovelace, and Sease (18) that this condition would correspond to a vapor-pressure lowering at 25° of 0.91 mm. Bouyoucos states¹ that his "unfree" water content is only slightly lower than the wilting coefficient for wheat, the value of which for the Greenville soil has been found to be 6.9, 8.0, and 8.2 per cent, respectively, with a mean of 7.7 per cent. These values were determined at different times during the summer of 1920, each number being the mean of 25 experiments. The discrepancies are due largely to the difficulty of deciding when the wheat was wilted. A vapor-pressure depression of 0.91 mm. corresponds to 6.4 per cent moisture in this soil.

¹In a later paper Bouyoucos states that the wilting coefficient corresponds more nearly with the -1.5° reading of his dilatometer than the -4° reading. This is in much better agreement with the results recorded above because a freezing-point depression of 1.5° would correspond with a vapor-pressure depression at 25° of 0.338 mm.

Moisture equivalent and vapor pressure

A complete solution of this problem is impossible at the present time on account of the lack of sufficient experimental evidence. It will be seen from figure 5, however, that the moisture equivalent corresponds to a vapor-pressure depression of about 0.02 to 0.04 mm. Unfortunately, on this portion of the curves the vapor pressure changes are very slight for large variations of the moisture content so that exact characterization of the curves can be obtained only when the precision of the vapor-pressure measurements reaches 0.001 mm.

A partial solution of the problem is outlined below. Since the centrifugal force of the machine tending to throw the water out of the soil is exactly counterbalanced at equilibrium by a negative moisture gradient, the observed "moisture equivalent" will decrease as the thickness of the soil layer in the centrifuge cup increases. Table 6 gives some observed values of the moisture

TABLE 6
"Moisture equivalents" of Greenville soil when varying amounts of soil were placed in cups
(Radius of centrifuge 153 mm.)

SOIL	THICKNESS OF LAYER	WATER		
		1	2	Mean
gm.	mm.	per cent	per cent	per cent
5	1.7	32.8	30.6	31.7
10	2.8	29.5	28.8	29.1
20	5.5	24.9	25.0	24.9
30	8.2	23.0	22.6	22.8
40	10.8	21.0	20.8	20.9
50	13.5	19.4	19.8	19.6
60	16.2	19.9	19.1	19.5
70	19.0	18.5	19.1	18.8

contents for the Greenville soil when varying amounts of soil were placed in the cups and centrifuged at 2460 revolutions per minute for 45 minutes.

The moisture determinations were made by drying the whole sample and therefore represent the values for the center of the blocks.

A correlation between these data and the vapor pressure can be found by making use of the potential function when it is remembered that the centrifugal force can be equated to the potential gradient:

$$\frac{d\psi}{dr} = r\omega^2 \quad (11)$$

where r is the radius of the machine to the element of the soil under consideration and ω is the angular velocity of the machine. On integration we get:

$$\psi = \frac{r^2\omega^2}{2} - \psi_c \quad (12)$$

where ψ_c is the value of the potential at the center of the centrifuge. This has not been determined, but the equation can be used with the data in table 6 by taking two values of r :

$$\psi_1 - \psi_2 = \frac{\omega^2}{2} (r_1^2 - r_2^2) \quad (13)$$

This equation can now be combined with equation (9) to give:

$$\psi_1 - \psi_2 = \frac{\omega^2}{2} (r_1^2 - r_2^2) = \frac{RT}{V_o} \frac{(\phi_1 - \phi_2)}{(\phi_o)} \quad (14)$$

From the above data the 70-gm. block of soil would contain 31.7 per cent of water in the outside 5-gm. layer, about 18.5 per cent at the center, and about 16 per cent in the inside 5-gm. layer. The calculated vapor-pressure depression from the outside to the center is 0.0148 mm. and from the outside to the inside 0.0288 mm.

The observed values in this region, though not determined in an altogether satisfactory manner, are from the slope of the curves, of this order of magnitude. Since the centrifugal force is 1000 times gravity it is interesting to observe that the vapor-pressure change in the centrifuge, as determined above, agrees closely with the change as calculated from a vertical column of water vapor, 1000 times the height of the soil column. The change of pressure in saturated water vapor at 25° is 0.00171 mm. per meter of elevation. A layer of soil in the centrifuge, 17.4 mm. thick, should give a vapor-pressure range of 0.0288 mm., while the corresponding vertical column, 17.4 meters high, should give a range of 0.0298 mm.

The influence of dissolved materials on vapor pressure of the soil

A study of this phase of the problem was originally the main purpose of this investigation. The concentration of the dissolved material is related to the vapor-pressure lowering by the well-known Raoult law:

$$\frac{p_o - p}{p_o} = \frac{N}{N_o + N}$$

where N represents the number of moles of solute in N_o moles of solvent. This relation is only applicable as it stands to "ideal" solutions and to dilute solutions of non-electrolytes. In the case of aqueous solutions of electrolytes approximate agreement is found by using the law, if instead of N we employ

$$(1 + ni)N$$

where n is the number of ions formed from one molecule of N and i the degree of ionization as found by the conductivity method. It has been shown, however, by Frazer and Lovelace (16, 18) that the molar depression of the vapor

pressure of potassium chloride is practically constant over the range 2.0M to 0.4M in spite of the fact that the conductivity indicates appreciable change in the amount of dissociation over this range. We are dealing, therefore, in the case of the soluble salts with complex thermodynamic relations to which Raoult's law and the other "laws of solution" do not apply rigorously, and for the complete solution of the problem from this point of view it will be necessary to measure the vapor-pressure depressions of the pure solutions of all the salts studied.

All of the thermodynamic properties of a solution are connected, however, by a set of equations into which the concentration of the solute does not enter. These "colligative properties" enable us to connect the freezing-point lowering, the osmotic pressure, the surface tension, the vapor-pressure lowering, etc. in a very satisfactory manner. Therefore, in the absence of vapor-pressure data for the common "alkali" salts we can make use of freezing-point data for purposes of comparing the observed depression of the vapor pressures of the soil with the depressions in the corresponding simple solutions.

The curves VII, IV, and VI in figure 5 illustrate the results that have thus far been obtained by adding equimolar amounts of sodium chloride, sodium carbonate, and sodium sulfate (0.3, 0.544, and 0.729 per cent, respectively, of the dry soil) to the Greenville soil. It will be noticed that the concentration of the soil solution should increase as the soil becomes drier.

The theoretical curve for sodium chloride (curve VII A) has been prepared from the data of Frazer, Lovelace, and Miller (16, 18) on the assumption that the vapor-pressure effects of the two salts are identical. This assumption seems justifiable because the molar freezing-point lowerings of the two salts are practically identical (Landolt-Börnstein tables). The agreement of the theoretical and experimental curves seems rather remarkable and indicates that there is only slight absorption of this salt, except at the region of maximum curvature, where the absorption increases to about 44 per cent.

The theoretical values for 0.544 per cent sodium carbonate have likewise been deduced from freezing-point data, but they are not shown in figure 5. They would be represented by depressions about 18 per cent greater than those shown in the theoretical curve VII, A. From this calculation it appears that the carbonate is in some way removed from the soil solution to the extent of 85 to 90 per cent. The absorption seems to increase as the soil becomes drier so that the curves approach rather than recede from each other as the moisture content decreases. The theoretical depressions for 0.729 per cent sodium sulfate would correspond closely with those given by sodium carbonate. The absorption indicated by this calculation therefore is about 70 per cent. The data are insufficient, however, to warrant the drawing of further conclusions at this time, but they indicate that it will be possible to secure much valuable information in regard to the concentration of the soil solution *in situ*, by further vapor-pressure studies.

SUMMARY

Part I

1. A method of measuring the aqueous vapor-pressure lowerings of soil, accurate to 0.01 mm. of mercury at 25°, is described and a few preliminary experimental results are given illustrating four different soils as well as the influence of adding the common "alkali" salts to one of them.

2. The vapor-pressure-moisture curves are shown to be rectangular hyperbolae over a wide range of moisture contents. This means that the vapor pressure is proportional to the reciprocal of the moisture content.

3. The position of each curve depends almost wholly on the texture of the soil in the absence of dissolved material.

Part II

4. Sodium carbonate, sodium sulfate, and sodium chloride are shown to be absorbed by the Greenville soil to the extent of about 85 to 90, 70, and 10 to 44 per cent, respectively, though the last two salts can be extracted completely.

5. Some of the energetic functions of these soils are analyzed in the light of vapor-pressure data and their significance pointed out from this viewpoint. Correlations are thus given between the vapor pressure and the following properties: hygroscopic coefficient, wilting coefficient, moisture equivalent, Bouyoucos' "solid water," capillary potential, surface tension, and curvature of the moisture surface. In the case of the moisture equivalent some independent data and calculations are given confirming a portion of the vapor-pressure data.

ACKNOWLEDGMENTS

The present work is a natural outgrowth of researches which have been going on for a long time in this laboratory under the direction of Dr. F. S. Harris on moisture, soil, crop and alkali relations. The author takes this opportunity of thanking Dr. Harris for continued assistance and encouragement during the progress of this work. He also desires to thank Dr. Willard Gardner for helpful criticism of the theoretical discussion in this paper. The mechanical analyses were painstakingly carried out by Dr. D. S. Jennings and Mr. H. E. Flanders. Messrs. Marion L. Harris, Karl Harris, Granville Oleson, Leslie Jenkins, and D. W. Pittman, and Mrs. Lilaine O. Thomas have assisted at various times with the experimental work.

The work is being continued, but it is hoped that the results from this method of attack will appeal to other investigators as being worth the labor involved and that vapor-pressure studies will take an important place in soil researches.

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THE INFLUENCE OF CERTAIN FERTILIZER SALTS ON THE GROWTH AND NITROGEN-CONTENT OF SOME LEGUMES

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Received for publication February 14, 1921

INTRODUCTION

Since the time of Hellriegel and Wilfarth (1888-90) who established the fact that symbiotic bacteria are responsible for nitrogen-assimilation by legumes, and that of Beyerinck (1888), who isolated the specific causal organism (*Bacillus radicicola*), the scientific world has believed that leguminous plants obtain the bulk of their nitrogen from the atmosphere. In recent years it has been fully demonstrated by a number of investigators also that calcium plays an important part in the soil in increasing the activity of this symbiotic organism and hence in stimulating the assimilation of nitrogen by legumes. It has, however, not been so fully shown just what fertilizing elements, other than calcium, and what combination or combinations of these elements best promote this nitrogen-assimilation and legume growth generally. To shed further light, if possible, on this somewhat obscure topic the investigation herein described was undertaken. In addition to the work of ascertaining the effect of certain fertilizer salts, containing the elements that it was thought fit to study specially, on the growth (dry-matter) and nitrogen-content of a few legumes, it was deemed advisable to investigate also the effect of these salts and of the resulting crop growth on subsequent soil nitrification.

HISTORICAL

It has been stated in the introduction to this thesis that in recent years it has been fully demonstrated that calcium plays an important part in the soil in stimulating symbiotic organisms and hence in promoting the growth of most legumes. Thus it was deemed advisable not to include lime in this investigation as a subject for further study but to provide each treatment, including that for the checks, with calcium carbonate. A mere reference here to the investigators of recent years who have found lime in various ways beneficial to legumes therefore must suffice. The list includes the following, in order according to the date of their published writings on the subject: R. Ulbricht (57); C. G. Hopkins (23); A. F. Khandurin (25); D. N. Prianisch-

nikov (46); T. L. Lyon and J. A. Bizzell (40); J. F. Duggar and M. J. Funchess (7); J. B. Abbott (1); J. G. Lipman, A. M. Blair, I. L. Owen, and H. C. McLean (36); Lipman, Blair, McLean and Wilkins, L. K. (37); Lipman and Blair (30, 31, 32, 33, 34, 35); W. Frear (14); F. W. Morse (43); E. B. Fred and E. J. Graul (15, 16); H. W. Truesdell (56); J. K. Wilson (60); C. R. Fellers (9). Doubtless there are other investigators who may be cited, but the experimental findings of the above-mentioned are adequate for purposes of establishing the fact that lime is beneficial in various ways to legumes as a whole.

The literature on the subject of the effect of various nutrient salts, other than calcium, on the growth and nitrogen-content of legumes, is not as extensive as that associated with lime and its effects thereon. Nevertheless, a search revealed a fair supply of published matter, particularly with reference to the action of individual elements, such as phosphorus and sulfur, on certain phases of the growth of legumes. This literature is cited below in chronological order within the citation of the published material in general appertaining to experiments with a particular nutrient element.

On the subject of the effect of nitrogen, in various forms, on the assimilation of atmospheric nitrogen and on the growth thereby of legumes, the following citations are furnished:

1910. Löhnis (39) discussed at length the earlier history of the investigations associated with this question. He cited numerous investigators and stated their individual contributions to this controversial topic, listing among the supporters of the idea of nitrogen-fixation by legumes in the presence of abundance of nitrogen, both organic and inorganic, Prazmowski, Beijerinck, Frank, Böhme, Aeby, Bäsler, Nobbe and Hiltner. On the other hand there were cited the names of investigators whose work in general favored the idea of non-fixation in the presence of strong nitrates, of ammonium nitrates or sulfates, of strong accumulation of nitrogen from continuous manuring, or of water cultures. These workers included Wohltmann and Bergené, Vines, Laurent, Nobbe and Richter, Mazé, Marchall, Flamand, and Hiltner.

1914. Lipman and others (30), by means of pot experiments, showed that there was little difference in the yield and nitrogen content of soybeans fertilized with varying quantities of acid phosphate, nitrate of soda, gypsum and calcium carbonate. Gypsum gave the lowest percentage of nitrogen. Calcium carbonate, nitrate of soda, or acid phosphate in double quantity did not affect the protein content of the plant appreciably, although this increased the yield.

1915 and 1916. Lipman and Blair (31, 32, 33, 34) found that the nitrogen content of soybeans increased with applications of nitrate of soda, ammonium sulfate and dried blood. They also found that in sand cultures nodule development was not depressed by nitrogenous fertilizers, and that therewith the yield of dry matter increased up to a maximum and then decreased.

1916. Shive (52) found salts, except in weak concentrations, injurious to soybeans grown in sand. Ammonium salts, other than ammonium sulfate, exerted a more toxic action on soybeans than any of the corresponding salts of potassium, sodium and calcium.

1917. J. K. Wilson (60) pointed out the effects of various salts on nodule development. In general, chlorides, phosphates, calcium compounds and carbon-containing compounds seemed to stimulate nodule formation, while sulfates and ammonia-containing fertilizers depressed this formation on soybeans.

1917. Truesdell (56) found that the use of nitrogen did not increase the number of nodules on alfalfa roots. Nitrogen had apparently a depressing influence on the air-dry weights of the first cutting of this crop, grown in uninoculated soil, but it had no harmful effects on subsequent cuttings. He also found that the addition of nitrogen to the soil increased the total nitrogen in the roots of alfalfa.

1918. Fellers (9) showed that nitrate of soda increased the yield of soybeans but inhibited nodule formation and consequent fixation of atmospheric nitrogen, and concluded that it is not economical to supply soluble plant-food in the form of nitrogenous fertilizers to this crop. Nitrate of soda caused an appreciable increase in the protein-content of soybean seeds.

1918. Hills (22) found that the presence of large amounts of potassium, sodium, and calcium nitrates proved detrimental to the formation of nodules on alfalfa. Alfalfa seedlings grown in the presence of large amounts of nitrate did not produce nodules when inoculated with a viable culture of *B. radiculicola*. Nitrates in soil cultures prevented the re-formation of nodules once removed and also caused a decrease in the number of nodules already present.

1920. Albrecht (2) concluded from his investigations that nitrogen fixation will take place in a soil containing large amounts of nitrogen in the form of either nitrates or organic matter, that no injurious effects on nitrogen fixation are caused by nitrates, that nodules are produced in the presence of large amounts of organic matter, and that variations in total nitrogen of a soil fail to affect nitrogen fixation.

On the effect of phosphorus upon the legume phenomenon, the following citations may be made:

1916. Shive (52), growing soybeans in solutions, found that phosphates caused injury to most of the seedlings where high concentrations of the radical PO_4 were employed.

1917. Truesdell (56) concluded that a part of the benefit to higher plants from phosphorus was due to some additional factor other than cellular stimulation and the quickening of soil bacterial processes, as suggested previously by Fred and Hart (17) and Lipman (29).

Working with Miami silt loam in earthenware jars under greenhouse conditions, Truesdell grew alfalfa with phosphorus (dicalcium phosphate) and phosphorus plus nitrogen (urea). The beneficial effect of phosphorus on

plant growth was noted almost from the start, and this rapid early growth may be accounted for, according to the author, only as a result of direct nutrition and stimulation of the plant by phosphorus, and as a result of the quickening of bacterial actions other than those connected with nitrogen fixation.

Phosphorus increased the formation of nodules, and this finding substantiated the previous investigations of Marchal (4), Laurent (26), Wohltmann and Bergené (61), Löhnis (38), Deherain and Demoussy (6), Flamand (10), Prucha (47), and J. K. Wilson (59).

Analyses of the roots of alfalfa showed an increase in the total nitrogen-content due to the addition of phosphorus.

The percentage of nitrogen in the first alfalfa cutting varied in inverse proportion with the dry-weights, this being in agreement with numerous observations that rapid-growing plants contain a smaller percentage of nitrogen, on dry-weight basis, than slow-growing plants. But here, though the phosphorus-treated plants grew faster than the controls, yet the total nitrogen was greater in this phosphorus-treated alfalfa.

Analyses of the third cutting, which was deemed more representative of the normal mature growth of the crop, showed an entire agreement between the results, due to phosphorus, obtained from the whole crop and from the first cutting, by way of increased total nitrogen and increased dry weight. But the third cutting showed an increase in the percentage of nitrogen in the tops for phosphorus treatment. Phosphorus caused a greater total of nitrogen and a greater percentage of nitrogen to be stored in the tops than did nitrogen treatment of the soil. The data for the inoculated and the uninoculated series agreed throughout. Consequently, the author concluded that the difference in the percentage of nitrogen must unquestionably be considered as resulting from phosphorus treatment. The results obtained by the use of phosphorus were (a) increased growth, and (b) greater efficiency in fixing and storing nitrogen. The nodule bacteria apparently had not only supplied more nitrogen to those plants that received heavier treatments of phosphorus, but had also stored a larger percentage of nitrogen in their tops.

The data seemed to indicate increased activity of root bacteria due to phosphorus, resulting in the above-mentioned benefits. This relation was especially evident in the third cutting where an additional benefit from phosphorus was expressed in the occurrence of an increased percentage of nitrogen.

1918. Fellers (9) concluded from field experiments with soybeans that the yields of total dry matter and seed are materially increased by small applications of acid phosphate, especially on well limed soils. One to two hundred pounds appeared to be as beneficial as large applications. He also found that nodule formation on soybeans was stimulated, on limed soils, by acid phosphate. The stimulation was not so marked on acid soils. This fertilizer seemed to exert a beneficial influence on protein formation in the seed on both limed and unlimed plots. The fertilizer treatment for soybeans that appeared to give the best return for the money invested was probably 200 to 400 pounds of acid phosphate, together with a ton of lime, per acre.

The intimate relation of potash to nitrogen-assimilation by legumes has in the past been definitely established by various investigators. Recent investigations on the subject of potash fertilizing of legumes however may be cited.

1918. Fellers (9), by field experiments, showed that muriate of potash in applications of 50 to 400 pounds per acre gave an average increase of about 10 per cent in the yield of total dry matter and seed of soybeans on both limed and unlimed plots. Nodule production was slightly stimulated on the limed plots but not on the unlimed. Potash, he found, had little influence on the protein content of the seeds of soybeans.

The literature on the subject of the effects of sulfur upon the growth and nitrogen content of legumes is fairly extensive, and from this published material the following citations may be made:

1911. Hart and Peterson (20) called attention to the apparent deficiency of sulfur in certain soils as related to the demands made upon this element by some species of agricultural plants, legumes included. Analyses of these crops showed alfalfa especially high in sulfur content, and that this crop's sulfur requirements were actually greater than the phosphorus requirements.

1912. Bernard (3) found crop increases from the use of sulfur.

1912. Boullanger (4) obtained increased yields of crops from the sulfur treatment of the soil.

1913. C. B. Lipman (27) concluded that gypsum stimulated the beneficial soil organisms on the roots of legumes.

1914. Lipman and Blair (30) fertilized soybeans grown in pots, with calcium sulfate, nitrate of soda and calcium carbonate at applications of 10 gm. and 25 gm. The maximum yield of soybeans for a single pot was obtained from the calcium sulfate treatment.

1914. Shedd (50) obtained beneficial effects from sulfate with various crops grown in soil cultures. There were decided gains in the growth of soybeans with applications of sulfur, ammonium sulfate, pyrite and ferrous sulfate and smaller gains with calcium, potassium, barium, magnesium, aluminum and sodium sulfates on a soil containing 600 pounds of sulfur and 3040 pounds of phosphorus per acre.

1914. Reimer (48) obtained increased yields of alfalfa grown in the presence of flowers of sulfur.

1915. Hart and Tottingham (21), by means of soil cultures in the greenhouse, found that sulfur in the form of calcium sulfate, more so than in the form of sodium sulfate, was beneficial to common red clover, especially lengthening its root-system, hence feeding power, and increasing the yield of the dry matter 23 per cent. They showed also increased yields of legumes with calcium sulfate added to a complete fertilizer over a complete fertilizer plus potassium chloride. Here, they claimed, the action of the calcium sulfate must have been direct.

The same investigators found that calcium sulfate was especially favorable in increasing the yield of grain in peas. Its effect in increasing straw was more in evidence with beans and red clover.

1916. Pitz (45) concluded that calcium sulfate in small amounts increased the yield of red clover and the formation of nodules. Sulfates stimulated the development of red clover bacteria as well as the young plant. Elemental sulfur, however, increased the yield of red clover but slightly, and did not affect the root development nor the formation of nodules.

1916. Duley (8) found that when used alone on silt loam soil, flowers of sulfur was beneficial to the yield of red clover. It also very markedly increased nodule production on the roots of red clover when added to a complete fertilizer.

1917. Shedd (51) grew soybeans, red clover, alfalfa, and other legumes with 100 to 200 pounds of flowers of sulfur. He found that in the soybeans, which showed an increased sulfur content, no corresponding increased protein content always was found. In five out of eight instances, however, soybeans grown in soil where sulfur was added showed an increase in the total weight of protein.

1917. Brown (5), from experiments conducted in the Hood River Valley of southern Oregon, states that sulfur is a valuable fertilizer for alfalfa, the sulfur content of which is very high, according to the experiment station analyses. There air-slacked lime failed to produce increased yields of alfalfa, but when followed by a 100-pound application of land plaster (calcium sulfate) at the end of the first cutting, the plants immediately took on renewed vigor and easily surpassed the unfertilized plot on a total season's yield by the end of the last, or third cutting. This increase was shown despite the fact that the first cutting showed 1168 pounds for the check versus only 480 pounds for the other. The experiments with flowers of sulfur did not show such large increases of alfalfa, and it would seem, stated the author, that the lighter applications are the most economical when applied each year. Sulfur being quite insoluble in water, hence not immediately available, it was recommended that it be applied in the fall or not later than January or February, whereas land plaster should be applied as early as March to produce good results.

1918. Tottingham (55) showed that the addition of sodium sulfate and calcium sulfate to the sulfur-free modification of Knop's solution, in amounts equivalent to the sulfur of the unmodified solution, produced a greater yield of dry tops of red clover than did the latter solution, calcium sulfate being very efficient in this respect. It appeared as if the sulfur of gypsum functioned in the molecular combination in which it was supplied. The data obtained indicated that a deficiency of sulfur supply restricts growth by limiting the synthesis of protein. The author stated that the more or less parallel fluctuations of the plane of sulfur supply, the weight of nitrogen assimilated, and the yield of dry tops of the red clover plants, indicated that sulfur deficiency restricted growth by limiting this synthesis of protein.

1919. Miller (42) concluded that the great increase found in the nitrogen content of the clover grown in soil where sulfate had been added, is the result, in all probability, of these sulfates stimulating the action of legume bacteria.

His experiments also showed that sulfates caused an increase in root development and in the number of nodules on the red clover roots.

1919. Reimer and Tartar (49) found that on various types of soil alfalfa and red clover were increased from 50 to 1000 per cent by the use of various types of fertilizers containing sulfur, gypsum included. The soils ranged from coarse granite soils to the heaviest adobes. None were acid nor noticeably alkaline. Fall applications gave best results. The sulfur fertilizers used were very stimulative of the root system, increasing its size and the number of nodules. The fertilized plants contained more sulfur, more protein, and more nitrogen than the unfertilized. Gypsum was equal to superphosphate in results, but it was expected that eventually the latter would give superior returns, because the phosphorus content of the soils experimented with was rather low. Rock phosphate gave negative results in this region.

1920. Stewart (54), from very slight increases in the yield of soybeans and alfalfa grown in the field, and from slight decreases in clover yields, over a period of years, concluded that sulfur is not a factor in the production of crops, on brown silt loam at least. After examining the results obtained with gypsum during a period of 18 years at the Ohio station, he concluded that it is quite evident that the apparently beneficial action of gypsum is due to its stimulating effect, particularly on bacterial life (shown by Greaves), thus enabling the crop to draw better upon the inadequate supply of phosphorus in the soil.

1920. Singh (53) found, by the use of pot cultures, that gypsum generally increased the process of fixation of nitrogen by *B. radiculicola*, the greatest increase occurring with the largest application. He further found that 1000 pounds of gypsum increased the yield of red clover, but that other applications did not have any effect on other legumes (alfalfa, Canada field peas, and soybeans). The nitrogen content of legumes, he found, was not affected by gypsum.

The literature upon the subject of the effect of fertilizer salts upon soil nitrification appears to be somewhat limited. A few citations having a bearing upon this phase of our investigation however may be stated.

1904. Fraps (11) pointed out that phosphoric acid and potash increased nitrification in some soils, while in other soils the opposite effect was produced.

1908. The same investigator (12) showed that these soil constituents had little effect upon the production of active nitrogen, though in some cases nitrification was affected considerably. With both phosphoric acid and potash the active nitrogen was much less affected than the production of nitrates.

1920. Fraps (13) also found that the addition of phosphate and of potash to potted soils increased nitrification in several types of soil and caused the soils which nitrify very slowly to nitrify in a shorter time. Dicalcium phosphate was more effective than potash (K_2SO_4) in these respects. He further showed that calcium carbonate increased nitrification. During these experiments, however, a considerable time elapsed before he noticed the formation of nitrates.

1909. Lipman (28) observed that the amounts of NO_3 nitrogen in parts per million were favorably affected by gypsum.

1912. Patterson and Scott (44) found that superphosphate increased nitrification of ammonia added to a soil, and concluded that this fertilizer may prove a useful aid to nitrification. The soil, however, was poor in P_2O_5 (0.032 per cent). They suggested that phosphates may help to nourish nitrifying organisms as well as the crop; and that where not required by these organisms, superphosphate, being acid, will probably do harm. Gypsum, they found, had a moderate effect in encouraging nitrification, but was not at all equal to calcium carbonate in this respect. They further showed that sodium chloride (salt) had a bad all-round effect on nitrate production.

1916. Jensen (24) found that bone meal, superphosphate, waste lime, and dry yard manure decreased the nitrifying activity in field soils. The manured plots lost most nitrogen, especially those to which ammonium sulfate was added, while the limed plots showed a gain in total nitrogen. Plots receiving calcium cyanamid, phosphatic fertilizers, and nitrate showed a slight gain in total nitrogen over the checks.

1916. Duley (8) showed that the nitrate content of the soil varied inversely with the amount of soluble sulfate in the soil.

1918. Fulmer (18) found that while nitrification is benefited by limestone, calcium carbonate and magnesium carbonate (particularly by the latter), it is only very slightly increased by phosphates (dibasic magnesium phosphate and monocalcium phosphate were used) in certain Wisconsin soils.

1918. Greaves (19) and his co-workers showed that calcium sulfate is more efficient than potassium chloride as a stimulator of nitrification, increasing nitric-nitrogen accumulation of the soil 97 per cent. They found that those compounds which are the strongest plant stimulants also are the most active in increasing nitric-nitrogen accumulation of the soil, and that it is very likely that the effect upon the plant is due mainly to the action of the compound upon the bacteria, which in turn render available more plant-food. They asserted, however, that the ammonifying powers of a soil containing alkalis are a better index to its crop-producing powers than are the nitrifying powers. They further found that nitrification was least with KCl out of the six chlorides experimented with. The soil, however, contained over 7 per cent of CaCO_3 , and therefore was suited for satisfactory nitrification results from the use of gypsum.

1920. Whiting and Schoonover (58), working with field soils in which soybeans were grown, showed that phosphorus in the form of rock phosphate increased nitric nitrogen to the extent of 18.09 to 19.01 pounds per acre, over and above that produced by organic matter (stable manure or crop residues).

1920. Singh (53), working with pot cultures, found that nitrification was depressed by gypsum alone, but the use of gypsum and lime together increased the process.

EXPERIMENTAL

Methods and results

Thirty-six square, stout wooden boxes were each filled with 128 pounds of a mixture composed of 110 pounds of clean sand and 18 pounds of a sandy loam soil. The soil medium was thus decidedly low in plant nutrients but contained enough to supply the crops grown provided it was in an available condition. This was designed to make very pronounced the effect of those fertilizer nutrients in the soil that were not readily available as compared with those that were. The inclusion of the loam served the purpose of introducing the nitrifying organisms. The subsequent crop growth was carried out in the greenhouse. The content of each box was compacted alike, and the moisture content of the soil, as far as possible, was maintained throughout at 10 per cent (on the dry-soil basis) by weighing the boxes at regular intervals, varying with the crop and with the stage of the growing season. On November 7 and 9, respectively, alfalfa and Canada field peas were each sown in 18 boxes containing 9 separate treatments, in duplicate. To each box was added $\frac{3}{4}$ pound of calcium carbonate, it having been shown by various investigators to promote assimilation of nitrogen by legumes. The varying treatments were as follows:

BOX NUMBER	TREATMENT
1, 10, 19, 28	No fertilizer (checks)
2, 11, 20, 29	Nitrogen (dried blood, 12 gm. per box)
3, 12, 21, 30	Phosphorus (disodium phosphate, 8 gm. per box)
4, 13, 22, 31	Potassium (muriate of potash, 8 gm. per box)
5, 14, 23, 32	Sulfur (gypsum, 8 gm. per box)
6, 15, 24, 33	Nitrogen, phosphorus, potash and sulfur in above forms (total 36 gm.)
7, 16, 25, 34	Nitrogen, phosphorus, and potash in above forms (total 28 gm.)
8, 17, 26, 35	Nitrogen, potash, and sulfur, in above forms (total 28 gm.)
9, 18, 27, 36	Phosphorus, potash, and sulfur, in above forms (total 24 gm.)

Previous to seeding, the boxes were inoculated with sand cultures containing the sub-species of *B. radiculicola* corresponding to the legume sown. In boxes 1 to 18 alfalfa was sown at the same rate as ordinarily sown under field conditions. The plants were subsequently thinned out to 23 per box. Boxes 19 to 36 were seeded with Canada field peas at the rate of 25 per box. These were later thinned out to 11 per box.

Because of backwardness in becoming established, due doubtless to an insufficient supply of nitrogen, the alfalfa seedlings were sprinkled on January 23, 1920, with a solution of nitrate of soda at the rate of 1.94 gm. per box (approximately 100 pounds per acre of 3,000,000 pounds of soil).

Five cuttings of alfalfa (cut when almost fully flowered, except in the case of cutting no. 5 which failed to flower because of the lateness and coolness of the season) were obtained. These were dried in the drying chamber, weighed and analyzed for dry matter and total nitrogen.

The peas, which produced an enormous growth, were carefully kept upright, and were harvested when fully ripe. The grain and straw were weighed, and analyzed for dry-matter and nitrogen, separately. Photographs of the pea growth are shown in plate 1.

Following the crop of Canada field peas, Ito San soybeans were seeded on May 22, 1920, after suitable inoculation of the soil. These were kept upright also and allowed to ripen fully before harvesting.* The grain and straw were weighed, and separately analyzed for dry matter and nitrogen.

The lime, in the form of calcium carbonate, was applied at the rate of 3 tons (of 2000 pounds) per acre of 3,000,000 pounds of soil, the salts at the rate of 282.6 pounds per acre, and the dried blood at the rate of 424 pounds per acre.

TABLE 1
Average total dry matter in the various crops for the various treatments

TREATMENT	ALFALFA (23 PLANTS, TOTAL OF 5 CUT- TINGS)	CANADA FIELD PEAS (11 PLANTS)				SOYBEANS (12 PLANTS)		
		Grain	Straw	Grain and straw		Grain	Straw	Grain and straw
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Lime alone (check).....	157.200	42.117	62.63	104.750	30.520	72.825	103.345	
Lime and nitrogen.....	154.935	53.480	74.49	127.970	30.970	74.525	105.495	
Lime and phosphorus.....	192.805	72.400	123.67	196.075	33.260*	88.920	122.180	
Lime and potassium.....	163.490	50.217	72.36	122.580	29.735	69.635	99.370	
Lime and sulfur.....	168.665	25.140	49.85	75.000	30.635	71.600	102.235	
Lime, nitrogen, phosphorus, potassium and sulfur.....	209.925	77.665	125.40	203.070	35.435	86.165	121.600	
Lime, nitrogen, phosphorus and potassium.....	197.690	77.250	130.62	207.870	32.205	85.940	118.145	
Lime, nitrogen, potassium and sulfur.....	174.995	32.677	56.54	89.220	28.520	73.135	101.655	
Lime, phosphorus, potassium, and sulfur.....	186.290	75.370	128.33	203.705	34.230	82.465	116.695	

* Only one box included in average.

Following the harvesting of the soybeans and of the fifth cutting of alfalfa, the boxes of soil (plus roots) were incubated for three weeks at greenhouse temperatures, the moisture content at 10 per cent being maintained throughout this period. Immediately following incubation, the contents of the boxes were carefully sampled by making six full-depth borings with a soil auger in each box. These samples were immediately extracted with distilled water and the extracts analyzed for nitrate nitrogen by the colorimetric method.

In the determination of total nitrogen in the various crops the Kjeldahl-Gunning method was used throughout. These determinations were conducted for the most part in duplicate, but where wide or reasonably wide variations between the duplicates occurred (as happened in a few instances, especially in analyzing the grain) triplicate determinations were made and the

nearest two titrations were selected for averaging. The dry-matter and nitrate-nitrogen determinations also were conducted in duplicate.

Tables 1, 2 and 3 show the average weights of dry matter and of the total nitrogen, also the average nitrogen percentages (based on the dry matter) for the duplicate boxes growing the three crops under all treatments.

Table 4 gives the averaged nitrification results from all salts, including lime (checks), after the growth of crops.

Tables 5, 6 and 7 record the percentage increases of dry matter, of total nitrogen, and of the percentage of nitrogen in the three legumes as the result of soil treatment with the above-mentioned nutrient salts. From these tables and tables 8 and 9 the conclusions enumerated at the close of this thesis have been drawn.

TABLE 2
Average total nitrogen in the various crops for the various treatments

TREATMENT	ALFALFA (5 CUT- TINGS)	CANADA FIELD PEAS			SOYBEANS		
		Grain	Straw	Grain and straw	Grain	Straw	Grain and straw
	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Lime alone (check).....	5.300	1.895	0.605	2.500	2.170	0.910	3.080
Lime and nitrogen.....	5.315	2.480	1.010	3.490	2.340	0.890	3.230
Lime and phosphorus.....	6.925	3.450	1.705	5.155	2.690	1.550	4.240
Lime and potassium.....	5.700	2.750	1.110	3.860	2.210	0.810	3.020
Lime and sulfur.....	5.645	1.235	0.860	2.095	2.240	0.815	3.055
Lime, nitrogen, phosphorus, potassium and sulfur.....	7.335	3.645	1.740	5.385	2.605	1.390	3.995
Lime, nitrogen, phosphorus and potassium.....	7.065	3.780	2.090	5.870	2.520	1.460	3.980
Lime, nitrogen, potassium and sulfur.....	5.930	1.560	1.055	2.615	2.105	0.995	3.100
Lime, phosphorus, potassium and sulfur.....	6.570	3.470	2.230	5.700	2.585	1.225	3.810

Tables 8 and 9 show the actual and percentage increases of nitrate nitrogen, in parts per million, after the growth of alfalfa and of Canada field peas and soybeans by the various nutrient salts. Dry-matter increases (actual) also are included for comparison with the corresponding nitrate-nitrogen increases.

During the growth of the legumes a few notes of special interest respecting the behavior of the plants were made from time to time.

In the peas the potash-treated plants were the first to flower, blossoms being noticed on the tall phosphorus-treated plants some two days later. Where potash was supplied the pods appeared to be best filled, while plants without a potash supply seemed insufficiently filled. Where a complete fertilizer was added the pods were more advanced and the vines ripened before those in the other boxes.

In the alfalfa the plants that received phosphorus flowered first and thereon the flowers were the most abundant. The accelerating effect of phosphorus on the reproductive parts of the crop was here demonstrated.

In the first growth of alfalfa an apparently injurious effect of sulfur was somewhat noticeable, but in later cuttings this was not visible. The inhibiting action on growth, more especially where sulfur was used alone, had disappeared, as is recorded in the percentage increases for the second and subsequent cuttings. On the other hand, this effect of sulfur used alone on the peas was visible throughout the growth of the crop.

TABLE 3
Average percentage of nitrogen in the various crops for the various treatments

TREATMENT	ALFALFA (5 CUT- TINGS)	CANADA FIELD PEAS			SOYBEANS		
		Grain	Straw	Grain and straw	Grain	Straw	Grain and straw
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Lime alone (check).....	3.420	4.480	0.960	2.720	7.125	1.240	4.18
Lime and nitrogen.....	3.490	4.640	1.360	3.000	7.525	1.200	4.36
Lime and phosphorus.....	3.616	4.765	1.385	3.075	8.090	1.750	4.92
Lime and potassium.....	3.559	4.530	1.535	3.032	7.430	1.160	4.29
Lime and sulfur.....	3.415	4.825	1.795	3.310	7.305	1.145	4.22
Lime, nitrogen, phosphorus, potassium and sulfur.....	3.555	4.695	1.360	3.027	7.335	1.610	4.47
Lime, nitrogen, phosphorus and potassium.....	3.611	4.890	1.610	3.250	7.845	1.710	4.78
Lime, nitrogen, potassium and sulfur.....	3.479	4.825	1.870	3.347	7.375	1.355	4.36
Lime, phosphorus, potassium and sulfur.....	3.600	4.600	1.735	3.167	7.555	1.485	4.52

TABLE 4
Average nitrate nitrogen in the soil after removal of crops, for the various treatments

TREATMENT	NO ₂ IN DRY SOIL		REMARKS
	After alfalfa	After Canada field peas and soybeans	
	<i>p. p. m.</i>	<i>p. p. m.</i>	
Lime alone (checks).....	4.95	10.7	Nitrification determina- tions after Canada field peas and soybeans, grown successively, had the advantage of the well rotted root system of the pea crop
Lime and nitrogen.....	6.85	11.4	
Lime and phosphorus.....	10.85	13.2	
Lime and potassium.....	4.50	7.4	
Lime and sulfur.....	5.85	8.2	
Lime, nitrogen, phosphorus, potassium, and sulfur.....	8.80	15.7	
Lime, nitrogen, phosphorus and potassium	13.65	22.5	
Lime, nitrogen, potassium, and sulfur....	11.55	10.5	
Lime, phosphorus, potassium and sulfur...	8.55	18.0	

Discussion of crop results

Upon referring to tables 1 to 3 and 5 to 7, it will be at once noticed that phosphorus has produced the most marked effect of all of the elements applied. The effect of phosphorus in increasing the dry matter, total nitrogen, and also, although to a lesser extent, the percentage of nitrogen in the legumes grown, is unmistakable. The literature cited above substantiates these find-

TABLE 5
Percentage increases of total dry matter over the checks, due to various treatments, for the three legumes*

TREATMENT	ALFALFA (TOTAL OF 5 CUTTINGS), PER CENT INCREASE	CANADA FIELD PEAS (GRAIN AND STRAW), PER CENT INCREASE	SOYBEANS (GRAIN AND STRAW), PER CENT INCREASE
Nitrogen.....	-1.504	22.167	2.080
Phosphorus.....	22.571	87.183	18.225
Potassium.....	3.935	17.021	-3.847
Sulfur.....	7.225	-28.401	-1.074
Nitrogen, phosphorus, potassium and sulfur....	33.455	93.861	17.664
Nitrogen, phosphorus and potassium.....	25.677	98.443	14.321
Nitrogen, potassium and sulfur.....	11.249	-14.826	-1.636
Phosphorus, potassium and sulfur.....	18.429	94.467	12.917

* Checks received lime alone and all treatments contained lime at the same rate.

TABLE 6
Percentage increases of total nitrogen over the checks, due to various treatments, for the three legumes

TREATMENT	ALFALFA (TOTAL OF 5 CUTTINGS), PER CENT INCREASE	CANADA FIELD PEAS (GRAIN AND STRAW), PER CENT INCREASE	SOYBEANS (GRAIN AND STRAW), PER CENT INCREASE
Nitrogen.....	0.283	39.6	4.870
Phosphorus.....	30.660	106.2	37.662
Potassium.....	7.547	54.4	-1.948
Sulfur.....	6.509	-16.2	-0.812
Nitrogen, phosphorus, potassium and sulfur....	38.396	115.4	29.707
Nitrogen, phosphorus and potassium.....	33.301	134.8	29.202
Nitrogen, potassium and sulfur.....	11.886	4.6	0.649
Phosphorus, potassium and sulfur.....	23.962	128.0	23.701

ings with respect to the beneficial influence of phosphorus; and these results add further testimony to the importance of this vital substance to the growth of crops and to the growth of leguminous crops in particular. Doubtless, this decidedly beneficial influence is due mainly to the bacterial stimulus by phosphorus, as is indicated by Truesdell (56).

With legumes, this experiment has indicated that any fertilizer, possibly with the exception of sulfur, that increases yield increases the percentage of nitrogen.

Naturally, combined nitrogen is not as essential to legumes as is phosphorus. Nevertheless, we find it playing some part in the growth of these crops, varying with the crop and its habit of growth and with the association of elements in which nitrogen is employed. For example, of the three plant species, peas were benefited in growth the most by nitrogen when it was used alone, while alfalfa was the least benefited; whereas by nitrogen in combination with other substances alfalfa was benefited in growth the most, and peas the least. While nitrogen, used alone, here slightly increased the percentage of nitrogen in the three legumes, particularly in the case of peas, yet when used in combination with other substances it did not have this effect.

In general, combined nitrogen in this experiment appeared to play some part in promoting nitrogen assimilation by legumes. It at least did not hamper the operation of this phenomenon, in keeping with the findings of the

TABLE 7
Percentage increases of percentage of nitrogen in plants over the checks, due to various treatments, for the three legumes

TREATMENT	ALFALFA (AVERAGE OF 5 CUTTINGS), PER CENT INCREASE	CANADA FIELD PEAS (GRAIN AND STRAW), PER CENT INCREASE	SOYBEANS (GRAIN AND STRAW), PER CENT INCREASE
Nitrogen.....	2.046	10.294	4.306
Phosphorus.....	5.731	13.051	17.703
Potassium.....	4.064	11.489	2.631
Sulfur.....	-0.146	21.691	0.957
Nitrogen, phosphorus, potassium and sulfur....	3.947	11.305	6.937
Nitrogen, phosphorus and potassium.....	5.584	19.485	14.354
Nitrogen, potassium and sulfur.....	1.725	23.069	4.306
Phosphorus, potassium and sulfur.....	5.263	16.452	8.134

majority of the investigators cited under this section; but whether or not the action would be impaired in the presence of large quantities of nitrogen is not within the scope of this investigation to answer.

The treatments were so arranged that only the effects of potassium used alone can be considered and these effects are beneficial in the cases of the growth of peas and of alfalfa, but apparently not in the case of the growth of soybeans. Peas were the most benefited in growth by muriate of potash, for this crop, of all three crops, showed the largest percentage increases of dry matter, of total nitrogen, and of percentage of nitrogen with potassium treatment.

Sulfur, without other fertilizer substances and in the form of gypsum, was apparently toxic to peas and slightly toxic to soybeans. To alfalfa, however, it proved beneficial, and this effect increased with the development of the crop, as shown by the successive cuttings, doubtless because of the disappearance of the toxic influence at first established in the soil. Had a less sandy soil been used the seemingly toxic effect noted, in all probability, would have

been less in evidence. Sulfur in combination with other substances was apparently toxic only in the case of peas, and even here this seeming toxicity was less marked than it was where sulfur was used alone. The fact that the treatments contained lime in fair quantity may possibly have accounted, in no small measure, for the satisfactory results obtained with alfalfa when fertilized with calcium sulfate—an experience recorded from experiments embodying the use of gypsum on calcareous soils.

As shown by Truesdell (56) in his investigations, the third cutting of alfalfa, on the whole, was the most satisfactory, the yields of dry-matter and the analyses being in general higher than those associated with the other cuttings.

The striking differences for the various treatments shown throughout the investigation have been made possible as the result mainly of using a compounded soil that was practically a sand. Had an ordinary soil been used, these differences would in large measure have been masked by the effect of plant-food elements inherent in the soil. The results herein obtained can at least lay claim to have in some small measure strengthened our knowledge of the growth requirements of legumes, and of alfalfa, Canada field peas and soybeans in particular.

TABLE 8
Increase in soil nitrification due to salts, after growth of alfalfa (5 cuttings)

INCREASE DUE TO	INCREASE OF NO ₃ OVER CHECK		INCREASE OF TOTAL DRY MATTER
	p. p. m.	per cent	gm.
Nitrogen.....	1.90	38.38	-2.365
Phosphorus.....	5.90	98.98	35.505
Potassium.....	-0.45	-9.09	6.190
Sulfur.....	0.90	18.18	11.365
Nitrogen, phosphorus, potassium and sulfur....	3.85	77.77	52.625
Nitrogen, phosphorus and potassium.....	8.70	175.75	40.390
Nitrogen, potassium and sulfur.....	6.60	133.33	17.695
Phosphorus, potassium and sulfur.....	3.60	72.72	28.990

TABLE 9
Increase in soil nitrification due to salts, after growth of Canada field peas and soybeans

INCREASE DUE TO	INCREASE OF NO ₃ OVER CHECK		INCREASE OF TOTAL DRY MATTER	
	p. p. m.	per cent	Peas and soybeans gm.	Peas alone gm.
Nitrogen.....	0.7	6.54	25.370	23.220
Phosphorus.....	2.5	23.36	110.160	91.325
Potassium.....	-3.0	-28.03	13.855	17.830
Sulfur.....	-2.5	-23.36	-30.860	-29.750
Nitrogen, phosphorus, potassium and sulfur....	5.0	46.72	116.575	75.100
Nitrogen, phosphorus and potassium.....	11.8	110.28	117.920	103.120
Nitrogen, potassium and sulfur.....	-0.2	-1.87	-17.220	-15.530
Phosphorus, potassium and sulfur.....	7.3	68.22	112.305	98.955

Discussion of soil nitrification results

A perusal of the soil nitrification results, as recorded in tables 4, 8, and 9, shows that salts or their combinations which most markedly promoted the growth of legumes usually caused the highest nitrification. Such was particularly the case wherever phosphorus was applied. This observation concurs with the conclusion of Greaves (19) who found that those compounds which are the strongest plant stimulants are also the most active in increasing nitric-nitrogen accumulation in the soil. He attributes this correlation to the stimulus given to the bacteria by the beneficial compound. This may be a factor in the results herein recorded, but we are inclined to give some recognition also to the effect of the decayed roots of the previous crop upon nitric-nitrogen accumulation. The increased top growth is correlated with increased root development, hence with more organic matter for nitrification. There was greater nitrification after peas and soybeans (grown in the same boxes of soil) than after alfalfa (five cuttings). The extensive root systems of the huge pea plants had opportunity to decay well, whereas there would be less decay of the alfalfa roots, even though extensive.

Nitrogen, applied alone, increased soil nitrification after all three crops, particularly after alfalfa; but when this nutrient was applied in combination with the other substances, it decreased nitrification after peas and soybeans and slightly increased it after alfalfa. It would thus appear that alfalfa is less dependent upon nitrate nitrogen for growth than are the other two legumes, peas especially.

Sulfur depressed nitrate-nitrogen accumulation, except when used alone as a fertilizer nutrient for alfalfa, which crop it also otherwise benefited, both alone and combined with other elements. In general, this finding was in accordance with the findings of Duley (8) who found that the nitrate-content of the soil varied inversely with the amount of soluble sulfate in the soil.

Potassium apparently slightly inhibited nitrate-accumulation after all three crops. It may here be mentioned, however, that because of the presence of chlorides (in the KCl used) there may possibly have been a slight loss of nitrates during the process of determination by the colorimetric method, which involves the use of pheno-disulfonic acid.

CONCLUSIONS

Effects of phosphorus

Of all the fertilizer elements in the salts applied to the compounded soil, phosphorus showed the most marked effect. As a single element it markedly increased the dry-matter and total nitrogen, and to a lesser extent the percentage of nitrogen in all three legumes, the order of greatest average influence on the crop being: (a) Canada field peas, (b) soybeans and (c) alfalfa. In the three crops phosphorus, used alone, showed its powerful influence on the three

factors in the following order: (a) increase of total nitrogen; (b) increase of dry-matter and (c) increase in the percentage of nitrogen.

In combination with nitrogen, potassium and sulfur, phosphorus markedly increased the dry matter and total nitrogen in Canada field peas, soybeans and alfalfa. However, it increased the percentage of nitrogen in soybeans and alfalfa only slightly, if at all, and decreased the percentage in the case of peas.

Effects of nitrogen

As a single element nitrogen can hardly be said to benefit the plants with respect to yields of either dry matter or nitrogen, or the percentage of nitrogen, unless in the case of Canada field peas, which appeared to respond somewhat in each of these three properties.

In combination with phosphorus, potassium, and sulfur, nitrogen promoted no more response in the legumes than where it was employed alone. Indeed, there was perhaps less response from nitrogen when used in association with the other elements.

Combined nitrogen did not hamper the operation of nitrogen assimilation by legumes; but whether or not it would have hindered the phenomenon had large quantities of nitrogen been used, could not be answered by this experiment.

Effects of potassium

Potassium, used alone, showed its greatest influence in increasing, on the average, the total nitrogen and dry matter in Canada field peas and alfalfa, in the order named. In soybeans, however, it showed a decrease with respect to these factors. Only in the percentage of nitrogen did potassium show an increase common to all three crops, and this in the crop order just named.

Effects of sulfur

Sulfur, in the form of gypsum, used alone and in combination with other fertilizer salts, increased somewhat the growth and nitrogen content of alfalfa but appears not to have had any effect on field peas and soybeans.

General effect of fertilizer salts

In general it may be said that when any application of fertilizer, with the exception of gypsum, increased the yield of the legumes grown, there was also an increase in the percentage of nitrogen in the plants.

Effects of fertilizer salts on soil nitrification, after legumes

Where phosphorus was applied there was, in general, the greatest nitrate accumulation after all crops. Thus salts or their combinations which most markedly promoted the growth of legumes, as did phosphorus, usually caused the greatest nitrification.

Nitrogen applied alone increased soil nitrification after all three crops, particularly after alfalfa, but when this nutrient was applied in combination with the other substances it did not have such an effect.

Potassium, in the form of muriate of potash, apparently slightly inhibited nitrate-nitrogen accumulation.

Sulfur, in the form of gypsum, increased nitrification in soil in which alfalfa had grown but not in soil in which peas and soybeans had grown. There appears to be a connection between the effect of sulfur on the crop and on nitrification following the crop.

In general, there appeared to be a tendency toward correlation between the dry matter produced and subsequent soil nitrification—due in part, it is assumed, to the greater root system associated with greater top growth, hence to greater amounts of decayed roots for promoting nitrification.

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PLATE 1

FIG. 1. Canada field peas fertilized with individual elements; note the pronounced effect of phosphorus.

FIG. 2. Canada field peas fertilized with elements in various combinations; note the pronounced effect of phosphorus.

INFLUENCE OF FERTILIZERS ON NITROGEN
CONTENT OF LEGUMES
ALEXANDER MACIAGGART

PLATE 1

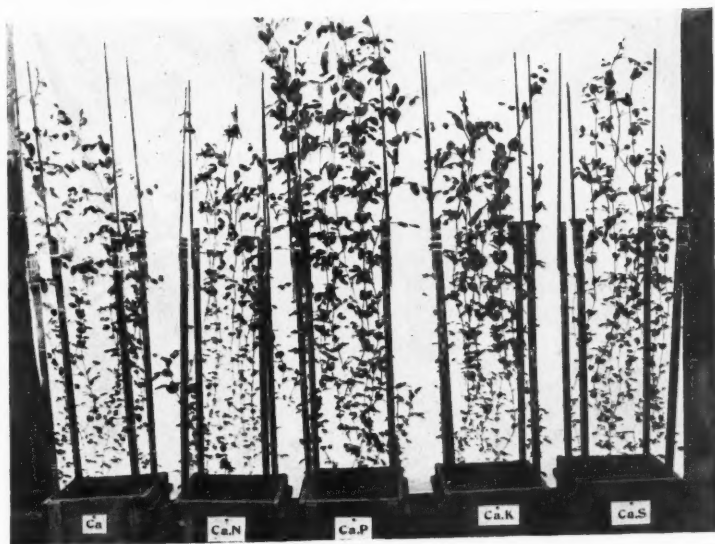


FIG. 1

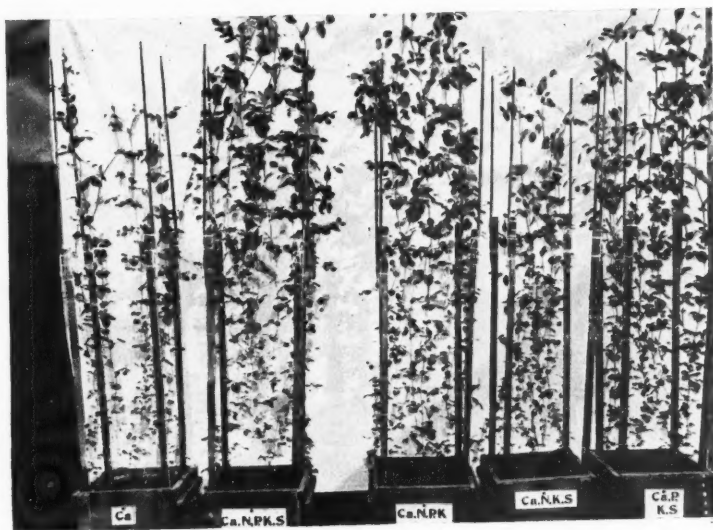


FIG. 2

1881
1882
1883
1884

STUDIES ON THE ACID AMIDE FRACTION OF THE NITROGEN OF PEAT. I¹

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Received for publication February 14, 1921

During the past decade a large amount of work has been done on the distribution of the organic nitrogen in soil organic matter. In this laboratory special attention has been paid to the composition of peat soils. The result of the work has been to show that "in comparison with ordinary proteins, peat contains a larger percentage of acid amide and humin nitrogen and a smaller amount of basic and non-basic nitrogen compounds" (7).

In a general way the total nitrogen in peat is divided as follows:

	per cent of total N
Humin nitrogen.....	2.0-8.0
Ammoniacal nitrogen.....	0.5-0.8
Acid amide nitrogen.....	10.0-20.0
Non-basic amino nitrogen.....	22.0-40.0
Non-basic non-amino nitrogen.....	3.0-10.0
Basic amino nitrogen.....	3.5- 6.0
Basic non-amino nitrogen.....	1.5- 6.5

It will be observed from these figures that the largest two fractions are the acid amide and non-basic amino or mono amino acid fractions. In pursuance of a plan outlined some time ago the former is now receiving special attention in our work.

Before proceeding further with the discussion it might be well to state just what is meant by the acid amide fraction as applied to peat. By this term is meant the nitrogen that is evolved as ammonia upon treatment with acid and subsequent distillation after making alkaline. When dealing with pure protein material the statement is justified that this truly represents the nitrogen present in the form of acid amides. It has been contended, and the authors admit the contention, that in a complex and little known material like peat it is not safe to apply the same criteria to the various subdivisions obtained that are used in the treatment of pure proteins. Hence it must be understood that the term "acid amide fraction" as used in this work refers to a fraction showing the same behavior that acid amides do in proteins, but that

¹ Journal article No. 18 from the Chemical Laboratory of the Michigan Agricultural College Experiment Station; published with the permission of the Director of the Experiment Station.

in peat it may be composed of other sorts of compounds. In other words, strictly speaking it is a term of convenience. It is true that there is no definite idea as to what these compounds might be and from the work of Jodidi (3) the preponderance of such evidence as we have is against their existence, but, nevertheless, in view of the uncertainty of our knowledge of the constitution of peat, it seems advisable to accept the possibility.

THE SIGNIFICANCE OF ACID AMIDES IN SOILS

The value of organic nitrogen compounds as sources of nitrogen for plant nutrition depends largely upon the ease with which they are converted into ammonia. From the readiness with which acid amides can be made to split off ammonia in vitro it would seem to be a logical conclusion that in the soil this fraction would be the one to become ammonified most rapidly, i.e. would be the most "available." An attempt was made to connect this fraction with the availability of nitrogen in peat as determined by the alkaline permanganate method but with small success, and the presence of such a large percentage of nitrogen in this form also affords an indication that it is not as easily decomposed as would be anticipated on the above grounds.

Actual experimental data on the behavior of acid amides in soils have been obtained by several investigators, notably Jodidi, Kelley and Lathrop.

Jodidi (3) added weighed amounts of amino acids and acid amides to definite quantities of soil and determined the ammonia produced. He found that the results depended upon the structure of the particular compounds used but that acid amides and amino acids did not differ materially as classes so far as the rapidity of their ammonification in soil was concerned.

Kelley (4) found that the basic nitrogen fraction of the materials investigated was more easily ammonified than either the non-basic or acid amide fractions. As regards the last two groups sometimes one and sometimes the other was decomposed more rapidly.

The most exhaustive work on the subject is that of Lathrop (5, 6), who studied the changes in the various fractions of nitrogen in dried blood when added to soil. He found that during the early stages the amide nitrogen diminished very rapidly. It then increased to a maximum after which it slowly decreased in quantity. The conclusion that Lathrop drew from his work was that through the action of microorganisms the dried blood was destroyed with the synthesis of a new protein which was much more resistant to decomposition than was the original dried blood.

Summarizing the results that have been obtained up to the present it may be said that soil organic matter contains material of a protein nature formed from the decomposition of protein which differs from the latter in having a high content of amide nitrogen compounds. These compounds differ from the acid amides ordinarily found in proteins in that they are very resistant to ammonification by the agents which are usually efficient in causing this. So

pronounced is this characteristic that these compounds may actually be produced in soils which exhibit a very active ammonifying power on the amides of ordinary proteins added to them.

The question which at once presents itself is, Why are the amides of soil organic matter so different from those of proteins in this respect? It has been shown that there is apparently a close relationship between the amide nitrogen contents and the amounts of the dibasic acids glutaminic and aspartic, which are present in proteins. The recent work of Dakin (1) who, in using Foreman's (2) method of separation, found that caseinogen contained a much larger amount of glutaminic acid than had formerly been obtained, will necessitate a revision of the theories on this subject, at least so far as this particular protein is concerned. His discovery of a third dibasic acid, hydroxyglutaminic acid, also tends to complicate the matter. Whether or not these same factors enter into the situation as respects other proteins is as yet unknown and in order to test it out in the case of soil organic matter which contains such an unusually large amount of amide nitrogen we are attempting to determine the relative amounts of the dibasic acids in peat.

That material of the nature of that under investigation contains some glutaminic and aspartic acids is known from the work of Suzuki (8) who isolated both from the hydrolytic products of humus by the esterification method. So far as we know, however, there has never been any attempt made to estimate them quantitatively.

In the course of the extensive work which has been carried on in this laboratory with peat soils, repeated attempts have been made to isolate glutaminic acid as the hydrochloride directly from the hydrolysates. The ease with which it can be obtained from the hydrolysates of many proteins naturally tempted us to try to get it from our material. The following experiments are typical of the methods of procedure employed and the results obtained.

Three hundred grams of oven-dried brown peat were digested with 1500 cc. of concentrated HCl for 16 hours on the hot plate. The product was filtered and the residue boiled with water and filtered through a Büchner funnel, the process being repeated several times. The combined filtrates were then concentrated to a volume of about 100 cc. by the method used in previous work. During this process crystals of calcium sulfate separated out and were removed by filtration. They were probably formed from sulfuric acid carried over from the drying flasks. The resultant solution was saturated with dry hydrochloric acid gas at 0° and allowed to stand some days in the refrigerator. A precipitate of inorganic matter had separated out at the end of this time, which was removed and the filtrate concentrated to a thin sirup. It was again saturated with hydrochloric acid and placed in the refrigerator after being inoculated with crystals of glutaminic acid hydrochloride. A second crop of inorganic crystals resulted. These were removed, the filtrate and washings concentrated, inoculated and placed in the ice box but even on long standing no crystals separated.

It was apparent from this and similar experiments that either there was too little glutaminic acid present to be separated in this way or the conditions for its crystallization were not proper, as a result, perhaps, of the presence of some inhibiting substance. In this connection the following quotation from Foreman (2) is of interest:

The ease with which glutaminic acid hydrochloride separates seems to vary with the protein under treatment and with the amount of glutaminic acid present. Plimmer (1912) states that separation of the hydrochloride "occurs in the case of caseinogen and certain vegetable proteins which contain from 10 to 40 per cent of this amino acid." If the protein contains under 10 per cent, then as a rule, no hydrochloride will separate. Occasionally, however, a separation is obtained; thus Hopkins and Savery (1911) working with Bence-Jones protein obtained 8.05 per cent.

To shed some light on this point 100 gm. of oven-dried peat was mixed with 30 gm. of casein and boiled with 500 cc. of concentrated hydrochloric acid on the hot plate for 48 hours. The mass was filtered through asbestos and washed by repeatedly boiling the residue with water and sucking it dry on a Büchner funnel. The filtrate and washings were combined and concentrated at 50° until a sirupy consistency was reached. This sirup was saturated at 0° with dry hydrochloric acid gas and after inoculation with a few crystals of glutaminic acid hydrochloride was set in the ice box and allowed to stand several days. A precipitate formed which was filtered on asbestos and washed with cold hydrochloric acid. After one recrystallization from hydrochloric acid it was pure white and melted at 197° (uncor.). It weighed 3.97 gm., representing a yield of 13.2 per cent.

It was thus made apparent that with the relative quantities of peat and casein used fairly good returns of glutaminic acid could be expected on the basis of that contained in the casein alone. The indications were that the amount present in the peat must be small. An attempt was next made to determine approximately the limits of precipitation for glutaminic acid from hydrolysates of peat.

Ten grams of casein and 187 gm. of peat were hydrolyzed and worked up in the usual manner, the filtrate and washings being concentrated to a volume of 20 cc. As a control 10 gm. of casein were treated in the same manner. The hydrolysates were saturated with dry hydrochloric acid gas and allowed to stand in the refrigerator for 4 days.

The precipitate from the casein alone weighed 1.45 gm. and melted at 197.5°. A second crop of 0.033 gm. melting at 195° also was obtained. This yield compared well with the results of Osborne and Guest which were the highest obtained up to the time of this work. The conditions seemed to be favorable for the maximum precipitation of the acid.

The precipitate from the peat-casein mixture, however, weighed 8.12 gm. and was largely inorganic, containing the chlorides of iron, calcium and magnesium. It was dissolved in distilled water and boiled with a slight excess of sodium carbonate to precipitate the heavy metals. The precipitate was

filtered off and the filtrate acidified with hydrochloric acid. The greater part of the sodium chloride was precipitated by passing in dry hydrochloric acid gas without cooling the solution. The first crop of sodium chloride thus obtained carried with it no organic matter, but succeeding crops carried an increasing proportion of organic matter. When the glutaminic acid hydrochloride was obtained, however, it still held an appreciable quantity of salt.

The possibility of precipitating the inorganic chlorides first by omitting the inoculation with glutaminic acid hydrochloride was next attempted. Ten grams of casein and 200 gm. of peat were mixed, hydrolyzed and worked up in the usual manner. After concentration the extract was placed in the refrigerator without inoculation. Two crops of inorganic crystals were obtained. The mother liquor was saturated with hydrochloric acid gas, seeded with glutaminic acid hydrochloride and set in the ice box for a week. The precipitate which separated out was filtered, dissolved in distilled water and boiled with an excess of barium hydroxide to drive off ammonia. The excess barium was then removed quantitatively with sulfuric acid, the solution made up to a volume of 100 cc. and the amino nitrogen determined on an aliquot, with the following results: 1 cc. gave 1.72 cc. nitrogen at 23° and 742 mm.

This corresponds to 1.24 gm. of glutaminic acid hydrochloride, a yield slightly lower than that obtained from casein alone.

It was evident that with the quantities present in the last series of experiments considerable difficulty would be experienced in getting satisfactory yields of glutaminic acid by the direct method. Further attempts along this line of attack were discontinued without applying the results of the above work to peat alone because our attention was called to Foreman's method of isolating both glutaminic and aspartic acids which offered greater possibilities than did our own method.

Foreman's method depends upon the insolubility of the calcium salts of the dibasic acids in alcohol and has afforded very valuable results in the hands of its originator. Dakin has also used it to good advantage. As usual, in applying to peat a method which has been developed for use with pure proteins, difficulties were encountered which made deviations from the original procedure necessary. However, we were able to accomplish our purpose and have isolated both glutaminic and aspartic acids from peat. The technic has not yet been perfected to the point where conclusions regarding the quantitative relationships can be drawn, but the results are promising for the early accomplishment of this end.

Foreman found that upon adding an excess of lime to the hydrolysate of caseinogen, filtering it and adding alcohol to the filtrate, a precipitate was formed which consisted of the following fractions:

- (a) Glutaminic and aspartic acids.
- (b) A gummy substance very difficult to crystallize.

(c) A very small quantity of pigmented substance precipitated from the solution by silver sulfate solution.

(d) A substance precipitated by phosphotungstic acid.

The general method of procedure consisted in general in separating the hydrolysate into these fractions.

The first trial was made with a mixture of 10 gm. of casein and 200 gm. of peat, the same quantities used in the second set of preliminary experiments described above. The mixture was hydrolyzed by boiling with a liter of concentrated hydrochloric acid and worked up as in the preceding cases. The sirup resulting from the concentration of the filtrate and washings from the insoluble residue was taken up in about 400 cc. of water and treated with a cooled paste of calcium hydroxide. After shaking thoroughly the precipitate was filtered off and washed free from chlorides by repeated suspension in water and filtering. The combined filtrate and washings were concentrated *in vacuo* until a fairly concentrated but not sirupy solution was obtained. This was transferred to a 2-liter flask and alcohol (about 97 per cent) added in small portions at a time, the mixture being shaken thoroughly after each addition. A black sticky precipitate formed on the bottom and sides of the flask. It was filtered through a Büchner funnel and washed as thoroughly as possible with alcohol. After dissolving it in about 300 cc. of water the calcium was removed by means of oxalic acid. The chlorides and some of the coloring matter were then removed by means of silver sulfate solution. After filtering off the precipitate the excess silver was removed by passing hydrogen sulfide through the solution. The filtrate from the silver sulfide was concentrated *in vacuo* until all of the hydrogen sulfide had been removed. Phosphotungstic acid was then added until precipitation was complete. The solution at this point was still strongly colored. After filtering off the precipitate the excess phosphotungstic acid was removed by means of barium hydroxide and the excess barium in turn quantitatively removed by means of sulfuric acid. The filtrate from the barium sulfate was concentrated as far as possible by distillation *in vacuo* and then transferred to a weighed evaporating dish and dried over phosphorous pentoxide.

In the dry state the residue weighed 11.068 gm. and contained a large quantity of inorganic matter. It was dissolved in water, treated with an excess of barium hydroxide, filtered and the excess barium removed as before. This time the dried residue weighed 9.4907 gm. It was a hard gritty mass and very difficult to triturate with glacial acetic acid which was the next step in the process and required most of a day. The acetic-acid-insoluble residue when dried weighed 4.4055 gm. It was dissolved in water and made up to a volume of 200 cc.

The acetic-acid-soluble part was freed from acetic acid by distillation *in vacuo*. The gummy residue was dissolved in water and made up to a volume of 50 cc.

Then 100 cc. of the solution of the acetic-acid-insoluble part was boiled with an excess of copper carbonate. The filtrate from the excess copper

carbonate was concentrated *in vacuo* and from it on standing overnight was obtained a large crop of fine light blue needles. These were filtered from the mother liquor which was further concentrated but it yielded no more crystals. Two crops of crystals were obtained on recrystallizing the product from hot water. They weighed, respectively, 0.2917 and 0.1055 gm., making a total of 0.3972 gm. from the 100 cc. used, or 0.7944 gm. from the total sample of peat casein mixture. This corresponds to 0.5420 gm. of aspartic acid.

After removing the copper by means of hydrogen sulfide from the filtrate from the copper aspartate and distilling off the excess hydrogen sulfide *in vacuo*, the residue was dried in a desiccator over sulfuric acid. It weighed 1.9211 gm. This residue was boiled with concentrated hydrochloric acid, cooled and saturated with dry hydrochloric acid gas, after filtering off the brown precipitate that formed during the boiling with hydrochloric acid. After inoculating with a few crystals of glutaminic acid hydrochloride the solution was allowed to stand in the refrigerator for 3 days. The perfectly white crystals were filtered on asbestos. They weighed 0.6234 gm. and melted at 197°, being apparently quite pure. A second crop of less pure product was obtained, which after treatment with animal charcoal melted at 196° and weighed 0.0307 gm. The total amount of pure glutaminic acid hydrochloride in the hydrolysate of the peat-casein mixture calculated from these yields was 1.31 gm., corresponding to 1.05 gm. of glutaminic acid. This, of course, does not include that in the mother liquors from the original crystallization and from the recrystallizations. These solutions were transferred to a 100-cc. flask and made up to volume. An analysis for amino nitrogen gave the following results: 1 cc. gave 1.84 cc. nitrogen at 23.5° and 749 mm.

The total amino nitrogen content based on these figures was 0.1017 gm. for the 100 cc., or 0.2034 gm. for the whole hydrolysate. This corresponds to 2.66 gm. of pure glutaminic acid hydrochloride. Undoubtedly there was much glutaminic acid present, but as no attempt was made to remove the ammonia before making the above analysis the figures are not entirely accurate.

The next step was to try out the method on peat alone. As over half of the above quantities of glutaminic and aspartic acids could be accounted for by the presence of the 10 gm. of casein, it was decided to use a sufficient quantity of peat to insure getting measurable amounts of these compounds. Accordingly, 3 kgm. of peat was hydrolyzed as usual with a solution of 9 liters of concentrated hydrochloric acid and 6 liters of water, this giving approximately a constant boiling mixture. The resulting hydrolysate was concentrated *in vacuo* to about a liter, cooled and saturated with dry hydrochloric acid gas. Three crops of inorganic crystals were obtained, the last mother liquor being diluted to about 2 liters and treated with a cooled paste of calcium hydrate. About 125 gm. of CaO were required to give an excess. The large gelatinous precipitate produced was washed free from chlorides and the filtrate and washings concentrated *in vacuo* to about 500 cc. As the

resulting solution was rather viscous 100 cc. of water was added to it and then alcohol in small portions. A black, sticky precipitate was formed and was not completely thrown down by 5 liters of alcohol. However, at this point it was filtered off and the alcohol distilled from the filtrate, which was then further concentrated. Alcohol was then added to it, but precipitation was still incomplete when 5 liters had been added. The precipitate, however, was filtered off at that point, the alcohol distilled from the filtrate and the liquid again concentrated. It was taken up in a small quantity of water and added in small amounts to comparatively large volumes of alcohol, the resulting precipitates being filtered through the same filter.

The precipitates were combined, washed as thoroughly as possible, dissolved in water and freed from calcium by means of oxalic acid. Coloring matter and chlorides were removed with warm silver sulfate solution, the excess silver precipitated with hydrogen sulfide and the filtrate concentrated *in vacuo*.

Phosphotungstic acid was then added until precipitation was complete, a large quantity being required. The excess was removed as usual by means of barium hydrate and the excess of the latter was precipitated quantitatively by sulfuric acid. The final filtrate was concentrated by distillation *in vacuo* and finally dried in a weighed casserole in a vacuum desiccator over sulfuric acid and P_2O_5 . The resulting product was a hard brittle mass weighing 91.8 gm.

Considerable difficulty was experienced in extracting this material with acetic acid, but finally after triturating it in a porcelain casserole with small portions of acid, 41 gm. of a grey powder was obtained.

The extract was concentrated *in vacuo* to a stiff gum and placed in a vacuum desiccator over KOH. Even after standing thus for 5 months it still smelled strongly of acetic acid.

SEPARATION OF GLUTAMINIC AND ASPARTIC ACIDS

The acid-insoluble grey powder contained 41.59 per cent of ash, which consisted apparently of oxides of tungsten, and 8.5 per cent of amino nitrogen figured on the ash-free basis. Two grams of it was dissolved in water, treated with barium hydrate to remove ammonia and the excess barium removed quantitatively with sulfuric acid. After treating with bone-black it was concentrated *in vacuo* and made up to approximately a liter. This solution was treated with an excess of copper carbonate and filtered. The filtrate and washings were concentrated to 500 cc. *in vacuo* and allowed to stand overnight. Light blue needles separated, and were filtered, dried and weighed. The yield was 0.4609 gm.

The filtrate was freed from copper and concentrated *in vacuo* to about 15 cc. It was saturated in the cold with hydrochloric acid, inoculated with glutaminic acid hydrochloride and allowed to stand several days in the ice box.

A crop of 0.1248 gm. of a white crystalline compound melting at 197° was obtained. The above work was now repeated with 25 gm. of the acetic-acid-insoluble substance. From this amount of material 5.716 gm. of the copper salt of aspartic acid and 2.00 gm. of glutaminic acid hydrochloride were obtained. These figures are for the purified products and do not take into consideration the residues left in the mother liquors.

0.0556 gm. copper salt was dissolved and made up to 25 cc.; 5 cc. samples gave 1.45 and 1.46 cc. of nitrogen at 22° and 743 mm.; average per cent amino nitrogen found 7.16; theory 7.19.

A portion of this copper salt was converted into the free acid.

0.0593 gm. was dissolved in water and made up to 25 cc.; 5-cc. samples gave 2.31 and 2.28 cc. of nitrogen at 25° and 744 mm.; average per cent amino nitrogen found, 10.51; theory 10.52.

The glutaminic acid hydrochloride melted at 197.5° .

0.0904 gm. was dissolved in water and made up to 10 cc.; 2-cc. samples gave 2.55 and 2.58 cc. of nitrogen at 25° and 736 mm.; average per cent amino nitrogen found 7.62; theory 7.63.

inferred
(2)

Estimation of pyrrolidon carboxylic acid

Foreman (6) has suggested a method for estimating the pyrrolidon carboxylic acid present by taking advantage of the fact that on boiling with hydrochloric acid it is converted into glutaminic acid with a corresponding increase in amino nitrogen.

A portion of the gum obtained in the above acetic acid extraction was analyzed for amino nitrogen.

0.0564 gm. gave 4.01 cc. of nitrogen at 23.5° and 742 mm. This corresponds to 3.88 per cent, or 1.971 gm. of amino nitrogen in 50.8 gm. of gum.

A portion of this gum was dissolved in water.

1 cc. of this solution gave 6.19 cc. of nitrogen at 24.5° and 753 mm. corresponding to 3.413 mgm. of amino nitrogen.

A 5-cc. portion was saturated in the cold in a small glass bulb with hydrochloric acid gas, a condenser was attached and the solution boiled for 15 hours. The contents of the bulb were diluted to 25 cc. and analyzed for amino nitrogen.

A 2.5-cc. sample gave 3.42 cc. of nitrogen at 24.5° and 744 mm. This corresponds to 3.724 mgm. per cubic centimeter, or 9.12 per cent of the total amino nitrogen.

The original 50.8 gm. of gum contained 1.971 gm. of amino nitrogen. Hence there would have been for the whole quantity of gum a total increase of 0.1798 gm. corresponding to a content of 1.657 gm. of pyrrolidon carboxylic acid.

This estimation was checked by extracting the pyrrolidon carboxylic acid from the gum with absolute alcohol. A portion of the gum weighing 12.65

gm. was triturated with absolute alcohol. The gum obtained by boiling off the alcohol was dissolved and made up to 50 cc. Of this 4 cc. was diluted to 25 cc.

1 cc. gave 0.72 cc. of nitrogen at 23.5° and 742 mm., corresponding to a total amino nitrogen content of 0.1228 gm. for the 12.65 gm. of the original gum.

Five cc. were hydrolyzed as before and made up to 25 cc.

1 cc. gave 1.30 cc. of nitrogen at 23.5° and 742 mm. This corresponds to a total of 0.1774 gm. of amino nitrogen in the 12.65 gm. of gum, or an increase of 0.0546 gm.

This is equivalent to a content of 2.0214 gm. of pyrrolidone carboxylic acid in the original gum as compared with 1.657 gm. by the former determination.

A 40-cc. portion of the solution of the gum was saturated with dry hydrochloric acid and boiled for 16 hours. After filtering off a flocculent precipitate, treating with animal charcoal and concentrating *in vacuo* it was seeded with glutaminic acid hydrochloride and placed in the refrigerator. After standing a few days a small crop of crystals was obtained, which in melting point and appearance under the microscope appeared to be identical with glutaminic acid hydrochloride. While it is possible that this arose from glutaminic acid dissolved by the acetic acid, it is more likely that it was formed from the pyrrolidone carboxylic acid since treatment of the gum directly without previous hydrolysis failed to yield anything resembling this compound.

DISCUSSION

This preliminary work shows several things of interest. In the first place, it shows that both glutaminic and aspartic acids are obtainable from peat by the methods employed. It also indicates the presence of pyrrolidone carboxylic acid, a fact which, of course, would be expected if glutaminic acid were present.

It is also apparent that while Foreman's method is applicable in a general way, certain details must be changed if it is to give satisfactory results with peat. This statement is true regarding the application to peat of any method devised for pure proteins. The presence of a comparatively high content of inorganic material and of humic organic substances introduces complications which make it necessary to make more or less extensive changes. In the present instance, for example, it becomes advisable to concentrate the original hydrolysate and saturate it with hydrochloric acid gas in order to remove as much of the inorganic matter as possible. But even then subsequent precipitates with the various reagents, such as calcium hydrate, phosphotungstic acid and silver sulfate are abnormally large as compared with those obtained in working up pure protein material. It is correspondingly difficult to wash them clean and at best they probably carry down more or less organic material which is ordinarily soluble.

The separation of the aspartic from the glutaminic acid in the acetic-acid-insoluble residue was not as sharp as was hoped for. The copper aspartate seemed to crystallize out almost completely in the first crop, for a further concentration yielded a very small amount of the light blue needles. When the glutaminic acid had been removed as completely as possible, however, the residue was again worked up for copper aspartate and a considerable quantity obtained. Carrying out the separation in the reverse order yielded no better results, as the glutaminic acid hydrochloride failed to crystallize readily and a humus-like precipitate came down in its place. It is possible that there is present some hydroxyglutaminic acid which complicates matters at this point.

The work is being continued along the lines suggested by this preliminary investigation.

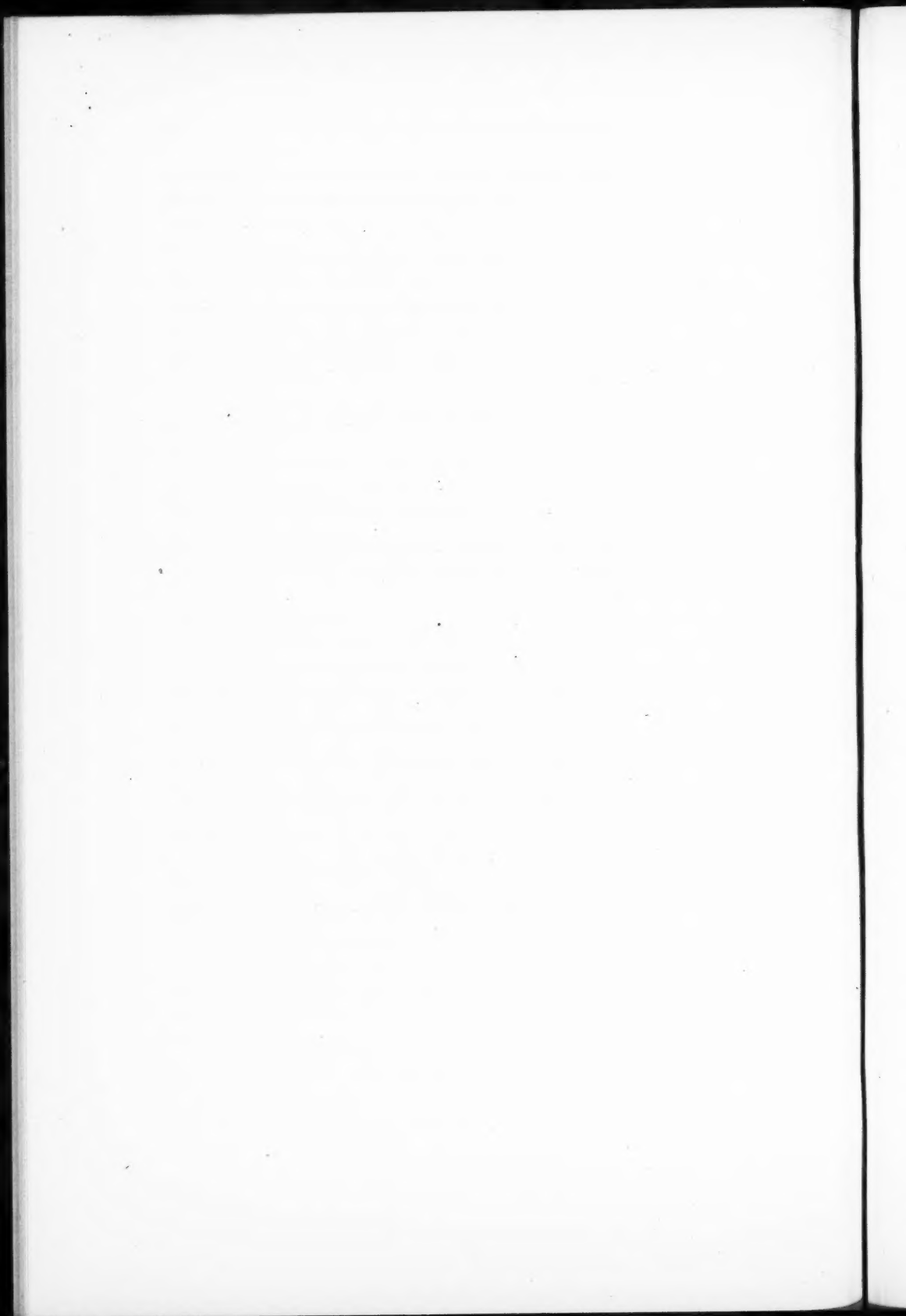
SUMMARY

Attempts to separate glutaminic acid directly from the hydrolysate of peat have failed.

The application of Foreman's method has resulted in the separation of both glutaminic and aspartic acids and the estimation of pyrrolidon carboxylic acid from this material.

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THE FIXATION OF ATMOSPHERIC NITROGEN BY INOCULATED SOYBEANS¹

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Received for publication February 21, 1921

Although it has been shown repeatedly by pot experiments that leguminous crops infected with the proper bacteria take nitrogen from the air there are much less data concerning the amount of nitrogen taken from the soil and from the air by plants grown under field conditions. With this in view, the Wisconsin Experiment Station has attempted to determine the amount of nitrogen taken from the soil and from the air by various leguminous plants. The usual method followed has been to compare the amount of nitrogen in infected plants with that in the same kind of plants grown on the same type of soil, but not infected. The difference between the amount of nitrogen in the inoculated and in the uninoculated crop represents the nitrogen secured by the cooperation of the bacteria with the higher plant. This difference in the amount of nitrogen fixed in the inoculated and the uninoculated crops is usually less than the actual difference which would result if it were possible to prevent altogether any infection from taking place in the uninoculated crop. The effect on the fertility of the soil planted to inoculated legumes is not brought out as well by analysis as by the growth of a non-leguminous crop on the same soil the following year.

In this article data are presented to show the amount of nitrogen taken from the air and from the soil when Ito San soybeans are grown under field conditions with and without the bacteria. The distribution of the nitrogen in the various parts of the plant, i.e., tops, roots, and nodules, also has been considered.

EXPERIMENTAL

The soil for this experiment was a light "blow" sand, low in plant food, especially nitrogen. For more than 20 years no leguminous crop had been grown here and no fertilizer had been added. In the spring of 1919 Ito San soybeans were planted in rows about 15 inches apart. One-half of the field was seeded with soybeans plus bacteria, the other half with soybeans minus bacteria. Except for inoculation, all the soybeans received the same treatment from the time they were planted until they were harvested. About 40

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days after the time of planting, the soybeans plus the bacteria showed a remarkable difference from the uninoculated in size and color. They were almost twice as tall as the plants without bacteria and a much darker green. Figure 1 of plate 1 shows clearly the difference in growth of the inoculated and uninoculated soybeans.

Representative portions from the inoculated and uninoculated plots were measured off and sampled. All of the plants, including roots and nodules, were carefully dug up from an area of 137.5 square feet. The nodules were carefully picked off the roots and brushed free of soil. The roots, tops, and, in the case of the inoculated plants, the nodules, were allowed to dry and were then weighed. Table 1 gives the results of these analyses.

TABLE 1
Yield of soybeans when grown with and without bacteria on Plainfield sand

TREATMENT	YIELD PER ACRE, AIR-DRY		
	Tops	Roots	Nodules
	lbs.	lbs.	lbs.
1. With bacteria.....	2598	197	115
2. Without bacteria.....	811	145	
Gain due to inoculation.....	1787	52	115

The figures show in a striking manner the effect of this partnership of soybeans and bacteria on the yield of forage. Soybeans with nodules produced more than three times as much dry forage as did those without nodules. The benefit of inoculation, however, is not confined to an increase in the yield of tops; there is also a gain in the weight of the roots. The plants were analyzed for total nitrogen and the data are given in table 2. Inoculation caused a decided gain in total nitrogen and also a gain in the percentage of nitrogen.

TABLE 2
Amount and percentage of nitrogen in soybeans when grown with and without bacteria on Plainfield sand

TREATMENT	AMOUNT OF NITROGEN PER ACRE				PER CENT OF NITROGEN		
	Tops	Roots	Nodules	Total	Tops	Roots	Nodules
	lbs.	lbs.	lbs.	lbs.	per cent	per cent	per cent
With bacteria.....	57.10	2.40	5.86	65.36	2.41	1.31	5.67
Without bacteria.....	7.46	0.87		8.33	1.02	0.65	
Gain due to bacteria.....	49.64	1.53	5.86	57.03	1.39	0.66	5.67

The percentage of nitrogen in the dried infected plant tops was 2.41, in the roots 1.31, and in the nodules 5.67, while the nitrogen in the dried uninoculated plant tops was 1.03 and in the roots 0.65. The point of chief interest in the figures of this table is the enormous increase in the amount of nitrogen

in the case of the inoculated plants. Soybeans from this plot with bacteria contained 65.36 pounds of nitrogen while soybeans from the plot without bacteria contained only 8.33 pounds of nitrogen. The amount of nitrogen in the treated plants was almost 8 times as great as that in the untreated plants. The growth of soybeans in partnership with the nodule bacteria resulted in a gain in the percentage of nitrogen in the plant and a gain in the total weight of the forage. Visible evidence of the value of inoculation on the yield of soybeans is given in plate 1 (fig. 2) and plate 2.

The amount of nitrogen taken from the air depends on many factors: completeness of inoculation of the crop, the type of soil, the rainfall, the temperature, etc. If the amount of nitrogen removed from the inoculated and uninoculated soils by the crop is equal, then the difference between these two figures gives the amount taken from the air. On the Plainfield sand this amount was 57.03 pounds for each acre, or in terms of protein, 356 pounds. Whether grown for feed or for fertilizer this high percentage of protein in the soybean is an actual gain to the farm. The inoculated soybeans have drawn more than 87 per cent of their nitrogen from the air.

THE RESIDUAL EFFECT OF INOCULATED AND UNINOCULATED SOYBEANS

The tops of the soybean plants were harvested. The roots of both crops were left in the soil; in the case of the inoculated roots this meant a great many nodules. The following spring these plots were seeded to rye. Here the effect of inoculation on the fertility of this soil is well illustrated. The rye on the soil which had grown inoculated soybeans the previous year produced a much more vigorous growth accompanied by a darker green color. This difference in growth and color between the inoculated and the uninoculated plots was clearly seen from a distance. Figure 1 of plate 3 is a photograph of this field showing a part of the inoculated and the uninoculated field. The variation in growth is apparent in the photograph, although this picture does not fully bring out the striking differences. A better idea of the difference in the growth of the rye is seen in figure 2 of the same plate.

SUMMARY

From the results of this field experiment with Ito San soybeans inoculated and uninoculated on Plainfield sand it may be concluded that:

1. Inoculation increased the yield of soybeans 1787 pounds per acre, or more than threefold.
2. Inoculation resulted in a net gain in nitrogen of approximately 57 pounds per acre. At the time of harvest, the inoculated soybeans contained 57.10 pounds of nitrogen in the tops, 2.40 pounds in the roots and 5.86 pounds in the nodules, while the tops of the uninoculated plants contained only 7.46 pounds, and the roots 0.87 pound. By far the greater part of the nitrogen

of inoculated soybeans, approximately 87 per cent of the total increase in nitrogen, is in the tops of the plants.

3. Aside of the gain in yield inoculation causes an increase in the percentage of nitrogen in the tops and roots.

4. Although the entire crop of soybeans, parts above ground, were cut and removed, the after effect of inoculation was clearly shown in the growth of rye the following year. The residue of soybean roots and nodules greatly benefited its growth.

PLATE 1

FIG. 1. Part of the inoculated and uninoculated plots of soybeans on plainfield sand.

FIG. 2. Soybeans from 137.5 square feet of the uninoculated and inoculated plots.



FIG. 1



FIG. 2

PLATE 2

TEN SOYBEAN PLANTS UNINOCULATED COMPARED WITH 10 PLANTS INOCULATED



PLATE 3

FIG. 1. Rye on plots which had grown soybeans the previous year; at the left the uninoculated plot, at the right the inoculated plot. The relative height of the rye may be judged from the persons in the field.

FIG. 2. Rye on plots which had grown soybeans the previous year; at the left 100 stalks from the inoculated plot, and at the right 100 stalks from the uninoculated plot.

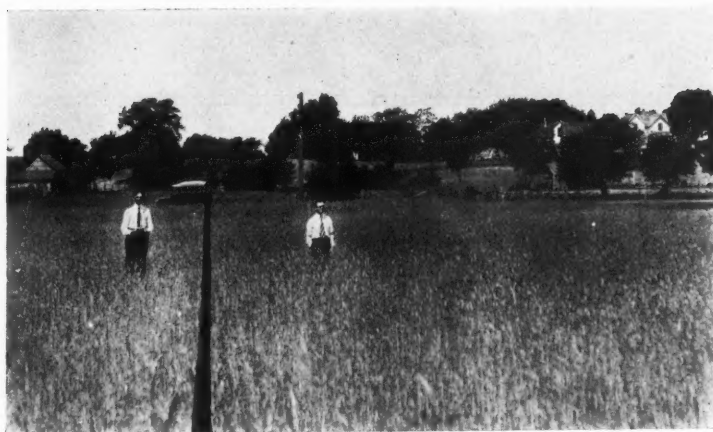
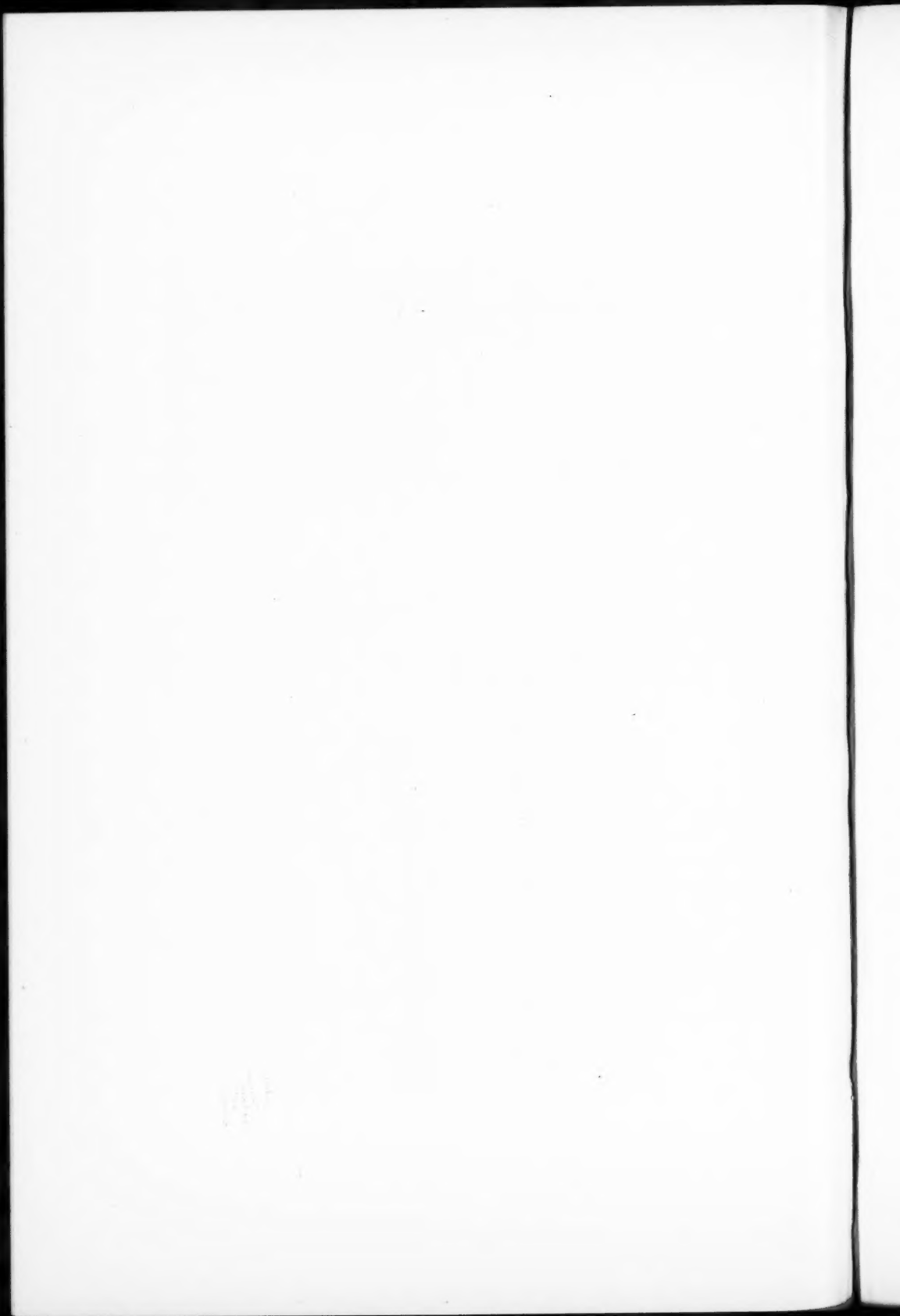


FIG. 1



FIG. 2



FIELD TESTS ON THE INOCULATION OF CANNING PEAS¹

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Received for publication February 21, 1921

INTRODUCTION

The results of field tests in 1918 with Alaska peas, inoculated and uninoculated, near Holmen, Wisconsin, showed clearly the beneficial effect of the legume bacteria. Although this poor sandy soil is not especially adapted to peas, it was found that well inoculated plants made a fair to good growth, while those without nodule bacteria were small with only 1 to 2 peas in a pod. This beneficial effect of inoculation under field conditions resulted in a more general use of cultures, especially in the neighborhood of Holmen. As might be expected, many of the farmers failed to find any noticeable gain in the growth of the inoculated plants. Apparently, in the fields where cultures failed to show any increased growth, the soil was either rich in available nitrogen or already well supplied with the legume bacteria.

It was found that in general inoculation of peas on poor sandy soil increased the yield; in rare cases a somewhat similar effect was noted on the rich loam soils. In the heavier clay types of soil no benefit was noted.

The Wisconsin Agricultural Experiment Station has studied some of the factors concerned with inoculation of peas, and some of the results are presented in this paper. The agents which influence the composition and yield of peas are of great importance. This is true both from the standpoint of peas for food and from the standpoint of peas in respect to soil fertility.

It was arranged in the spring of 1920 to investigate the effect of inoculation on peas grown in different sections of the state. The desire for more accurate information in regard to the nitrogen supply of peas prompted the analytical work. By means of total nitrogen analysis of the soil before and after growing a crop, as well as analysis of the pea plants, it is possible to measure the effect of peas on the nitrogen supply of soil. In order to get more exact data in regard to inoculation, special field plots of canning peas were grown in Chippewa and Dane counties.

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CHIPPEWA COUNTY

In coöperation with the "Big Four" Canning Company an experimental plot of 1 acre, near Stanley, was planted to inoculated and uninoculated peas. An area of heavy rich clay loam soil, slightly acid, sloping to the eastward and 5 by 32 rods in extent was selected. This plot was plowed and harrowed and then staked off into two equal strips with an unplanted strip 2 feet wide between. One of the half-acre plots was drilled with uninoculated Alaska peas and the other with the same variety inoculated. The plots were planted May 14.

At the time the peas were planted it was understood that this plot of land had never before been planted to peas, at least not in several years. However, several weeks after planting it was learned that the plot selected for peas had been planted to this crop for many years. There was a good development of plants over the entire plot, except for a small area where heavy rain drowned out about an equal number of peas in each section. The plot was examined on July 2 and an abundance of nodules found on the plants in both the uninoculated and inoculated parts.

No difference in size or color could be seen in the peas from the inoculated and uninoculated sections. The peas were harvested under careful supervision on July 12 and the plants from each part of the plot vined separately. The yield of shelled peas was as follows:

Inoculated.....	pounds 750
Uninoculated.....	780

The weights of the peas agree with the observations on distribution of nodules; there was no benefit from inoculation in this soil. The slight differences shown could easily be due to differences in soil, stand, or handling. On account of the total lack of field differences no analyses of plant tissue were made.

DANE COUNTY

The soil selected for this plot is a rich Carrington silt loam soil near Windsor. The Truog acidity test showed that the soil of this plot has a lime requirement of about 3 tons per acre. No lime was used in the experiment.

After it was plowed and harrowed the plot was divided into two equal parts $\frac{1}{2}$ acre in size. Each of the small plots was 7.5 feet wide and 115 feet long. The seed used was the commercial Alaska pea. Each of the small plots was sown in nine drills 115 feet long. Seed was sown at the rate of 4 bushels to the acre. On April 17 the plot was planted; uninoculated peas were sown first, then inoculated peas. The plants were examined on May 26 (full blossom), June 12, June 16 and June 21. On May 26 a difference in the size of the plants in the two plots was evident. By June 12 the inoculated peas were a darker green and the difference in size was more marked. The

peas were harvested on June 22. At this time the majority of the pods had reached the proper stage for harvest. The difference in the size and color of the plants in the plots is well shown in figure 1 of plate 1. The relative size of the yield of vines with the pods is shown in figure 2 of the same plate. Each pile is from one-fourth of the plot, or 0.01 acre. The yield of green vines and pods from the two plots was determined by weight and is given in table 1.

TABLE 1
Total green weight of vines and pods from inoculated and uninoculated peas

	AREA	
	0.01 acre	1 acre
	lbs.	lbs.
1. Inoculated	189	18,900
2. Uninoculated	157	15,700
Gain due to inoculation	32	3,200
Gain, percentage	20.37	

Comparison of weights shows that there were 3200 pounds per acre more green weight of vines and pods on the inoculated than on the uninoculated plot. This difference amounts to 20.37 per cent.

The pods were picked from the two piles of vines shown in figure 2, weighed, and the peas shelled and weighed. Table 2 gives the weights of the pods and peas from inoculated and uninoculated plants.

TABLE 2
Total green weight per acre of inoculated and uninoculated peas and pods

	PODS	PEAS
	lbs.	lbs.
1. Inoculated	3,880	2,570
2. Uninoculated	3,805	2,370
Gain due to inoculation	75	200
Gain, percentage	1.97	8.44

There was a substantial increase in yield in the inoculated plot in vines, pods, and peas.

The shelled peas were graded and the yields of the various grades are shown in table 3. From the figures of this table it will be seen that the peas from inoculated plants are larger than those from the uninoculated. The increase in size results in a decided gain in weight of peas belonging to grades 3, 4 and 5. The benefit of inoculation of this comparatively rich soil is of two kinds; first more of the large size of peas, due no doubt to greater maturity, and second, an increase of 8.44 per cent in total yield (table 2).

TABLE 3
Proportion of various grades of peas from inoculated and uninoculated plots

	YIELD PER ACRE		
	Grades 1 and 2	Grade 3	Grades 4 and 5
	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>
1. Inoculated.....	321	1,350	829
2. Uninoculated.....	466	1,111	691
Decrease or increase.....	-145	+239	+138

EFFECT OF INOCULATION ON NITROGEN SUPPLY OF THE SOIL

Chemical analyses of the soil from both parts of the plot were made for acidity (Truog test), for nitrates, and for total nitrogen. The determinations of nitrates and total nitrogen were made on an air-dry basis. In each case determinations were made in triplicate and total nitrogen determinations were made on two sets of samples. One set of soil samples was taken when the peas were harvested on June 24, and another on August 26.

Nitrates

In both parts of the plot, the inoculated and the uninoculated, the nitrates were present in such small amounts that it was not possible to make a colorimetric determination. Only a trace was present.

Total nitrogen

In order to compare the effect of inoculated and uninoculated peas on the total amount of nitrogen in the soil, careful analyses were made of the soil at the beginning and after the peas were harvested. At the time of planting the soil contained 260 mgm. of nitrogen in 100 gm. of dry soil. Because of the difficulty in drawing representative samples, portions of soil were taken from at least 10 different places in each of the plots and from these a composite sample prepared. The results of the nitrogen analyses are given on the dry basis. In each case the figures of table 4 represent the average of at least three to four closely agreeing analyses. Almost 2 months after the crop was harvested, soil samples from these two plots were taken and the analyses repeated. Here again, the soil of the inoculated pea plot gave a decidedly higher nitrogen content than that from the uninoculated plot. From the data it is seen that there is a marked residual effect of the inoculation. The results are calculated on the basis of a layer of soil 4 inches deep over one acre, or 1,000,000 pounds (Hopkins). It was deemed only just to calculate nitrogen on an acre 4 inches rather than on the acre 8 inches because; (a) most of the nodule formation, root growth, and hence nitrogen fixation takes place in this upper 4 inches and not in the 4 inches below, and (b) for samples

of soil as taken were from this upper 4 inches. It is evident from the results of these analyses for total nitrogen that with this type of soil and with proper inoculation there is no less nitrogen in the soil after a good crop of peas has been grown, cut, and removed, than there was before the planting. It must be noted that the plants from both inoculated and uninoculated plots bore nodules; although there were more and larger nodules on the inoculated plants. In both cases, the nitrogen content of the soil after removal of the crop was at least equal to the amount of nitrogen in the original soil. At the same time

TABLE 4
Effect of peas on the nitrogen supply of the soil

ANALYZED JUNE 24	TOTAL NITROGEN IN 100 GM. OF SOIL	TOTAL NITROGEN IN 1 ACRE OF SOIL
	<i>mgm.</i>	<i>lbs.</i>
1. Inoculated	276.1	2761.0
2. Uninoculated	262.9	2629.0
Gain due to inoculation	13.2	132.0

a gain in the nitrogen in the soil from the inoculated plot showed the benefit of good inoculation, produced by use of pure cultures, as compared with natural inoculation from the soil. A good crop of peas with a total green weight of 18,900 pounds per acre and a total weight of 2,570 pounds of shelled peas was grown and removed with an increase in the residue of nitrogen in the soil.

EFFECT OF INOCULATION ON THE NITROGEN CONTENT OF PEAS

Total nitrogen determinations were made of the two samples of pods from the Windsor plots. The results of the nitrogen analyses are given below:

	Nitrogen in 100 gm. of dry tissue
	<i>mgm.</i>
Inoculated pods	2355
Uninoculated pods	2333
Gain due to inoculation	22

It is evident from the figures not only that inoculation increased the yield of pods but that there was a corresponding increase in the nitrogen content. This increase in nitrogen content is important to the farmers who feed the pea-vine silage from the viner station, for it means: (a) that more of the valuable fertilizer, nitrogen, is returned to their farms, and, in part, to the soil and (b) that the silage is more valuable as a feed because of its increased nitrogen content. The gain in nitrogen through inoculation, then, cannot be measured simply by analyses of the soil after the peas are cut, but also must include the increase of nitrogen in pea silage. Not only are there more

pounds of silage per acre, but each pound contains more nitrogen than silage from uninoculated peas.

HORTICULTURAL GARDEN EXPERIMENTS

In order to test the effect of the inoculation of peas on very fertile soil, two rows of peas were planted in the truck garden of the horticultural department at Madison. These rows, 178 feet long and 18 inches apart, were planted on May 29 to Alaska peas. The soil is a heavy silt loam that has been heavily manured with stable manure for many years. The Truog test for soil acidity shows a neutral reaction. According to analyses this rich black garden soil contains about 0.410 per cent of nitrogen. The peas in the east row were uninoculated and the peas in the west row were inoculated.

The plants developed normally but the stand was much reduced by pigeons. In many places the peas were scratched out of the soil. About two-thirds of a stand remained in each row. On June 28, four weeks after planting, the plants started to form pods. At this time there was no apparent difference in size or color of the vines in the two rows. On July 13, almost 7 weeks after

TABLE 5

Green weight of 100 inoculated and 100 uninoculated pea plants from the horticultural plot

	VINES	PODS	PEAS
	gm.	gm.	gm.
Inoculated plants.....	552	394	158
Uninoculated plants*.....	360	133	145
Gain due to inoculation.....	192	261	13
Gain in percentage.....	53.33	196	8.96

* Many immature pods.

planting, about 25 representative plants were selected from each row. The uninoculated peas showed a few small nodules and on certain plants the roots were soft and black—perhaps because of “damping off” fungi. The inoculated plants showed a better root system and more and larger nodules than the uninoculated plants. Little if any disease could be found on the roots of the inoculated plants. From each of these two groups of plants ten were selected as an average to be weighed and photographed. The tops and roots are shown in plates 2 and 3. The weight of ten average uninoculated plants was 123 gm. and of ten inoculated plants 143 gm.

On July 15 the entire two rows were harvested and 100 plants were selected from each by a garden laborer who was not familiar with the plan of the experiment. He was asked to select 100 good plants from each row. These groups of plants were weighed and analyzed (table 5).

The effect of inoculation here is similar to that of the previous experiment. The greatest difference was noted in the vines and pods but there also was a marked difference in the yield of shelled peas.

Unfortunately the seeds of these peas were lost during drying. The analysis of the pods is shown below:

	Nitrogen in 100 gm. of dry tissue mgm.
Inoculated peas.	1853
Uninoculated peas.	1743
	<hr/>
Gain due to inoculation.	110

Because of the small area of peas used in this test no attempt was made to calculate the results in pounds per acre. It seems fair, however, to say that the results in general agree with those obtained at Windsor.

CONCLUSIONS

In the experiment at Stanley, with a heavy, rich, clay loam soil, slightly acid, which had been cropped to peas for years, inoculation apparently had no effect.

In the Windsor experiment with a rich silt loam soil, unlimed and acid inoculation has been shown to be beneficial. Inoculation has caused an increase in the total yield of pea plants, in the yield of peas, and in the percentage of nitrogen. An increase in the weight of shelled peas means an increase in cash returns. An increase in the nitrogen content of the plant means an increase in the value of the plants or pea silage as feed. Analyses of the soil in the Windsor plot have shown that together with an increased crop of peas there is a gain in nitrogen in the residual soil where inoculated plants have been grown, or that the inoculated peas have acted as an efficient nitrogenous fertilizer. Nitrogen has been added not only for the benefit of the pea crop itself but also for the subsequent crops on that soil.

The experiment on the Horticultural Garden plot has indicated that these favorable results of inoculation are not confined to the acid silt loam soil. The soil in this case was neutral in reaction, and had been heavily manured for years. Again inoculation produced an increase in yield and in the percentage of nitrogen in the plants.

PLATE 1

FIG. 1. Alaska Peas on Carrington silt loam soil. The signs are equally distant from the soil.

FIG. 2. Alaska peas on Carrington silt loam soil. The piles of peas have approximately the same diameter.

E. B. FRED, W. H. WRIGHT AND W. C. FRAZIER

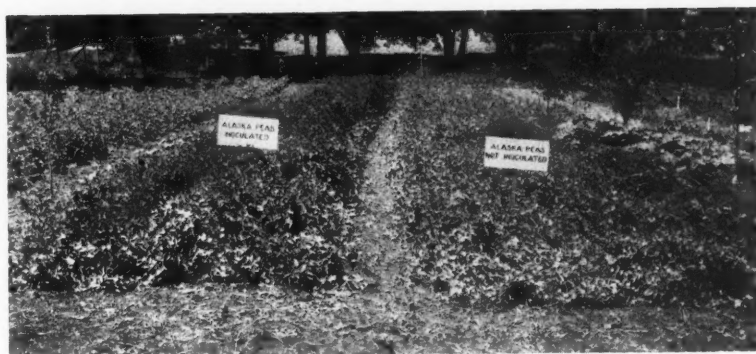


FIG. 1



FIG. 2

PLATE 2

PEAS FROM THE HORTICULTURAL PLOT AT MADISON, SHOWING ROOTS AND TOPS; INOCULATED
AND UNINOCULATED

FIELD TESTS ON THE INOCULATION OF CANNING PEAS

E. B. FRED, W. H. WRIGHT AND W. C. FRAZIER

PLATE 2

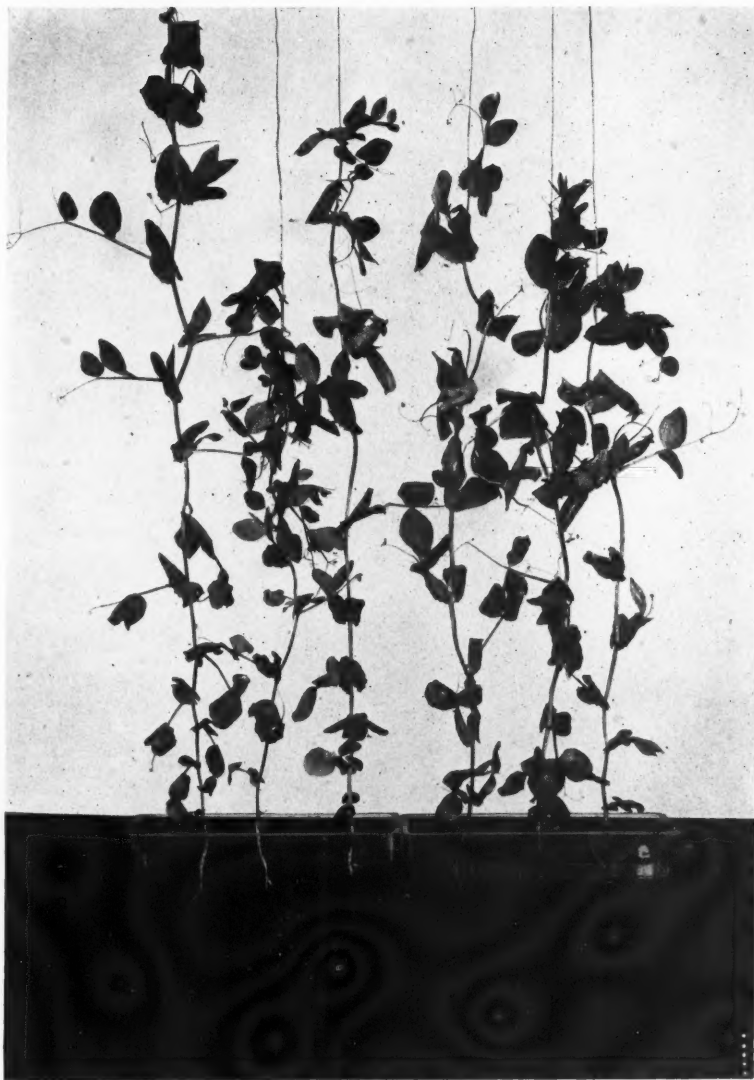
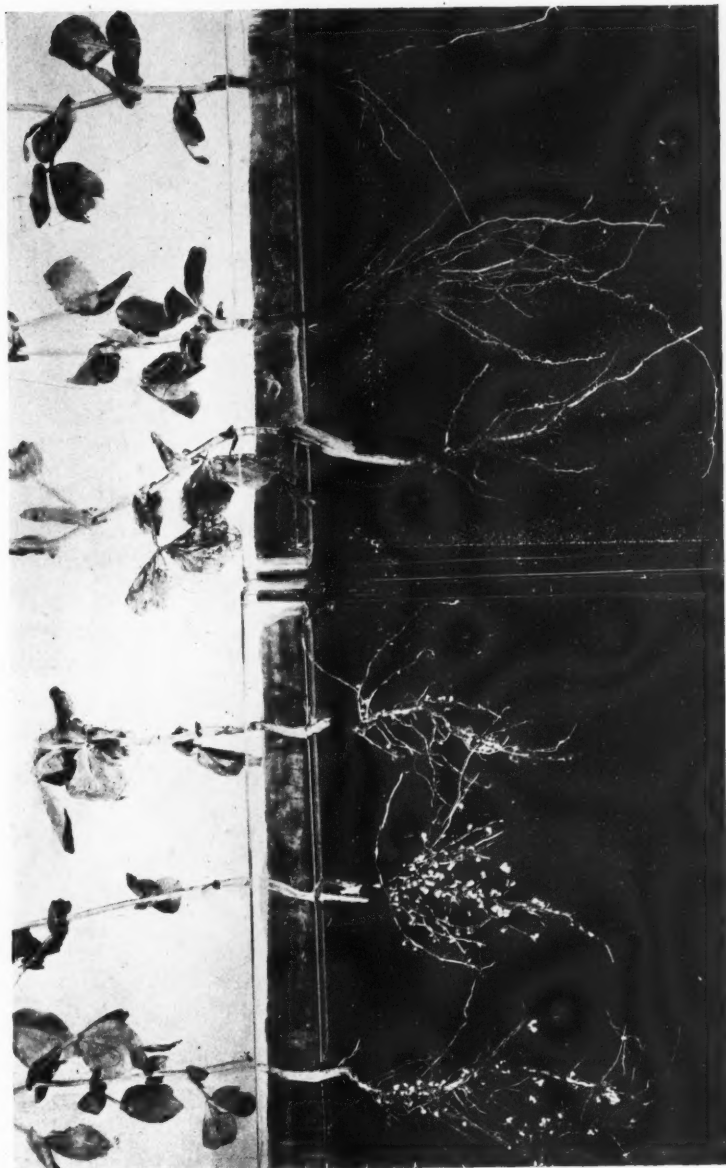


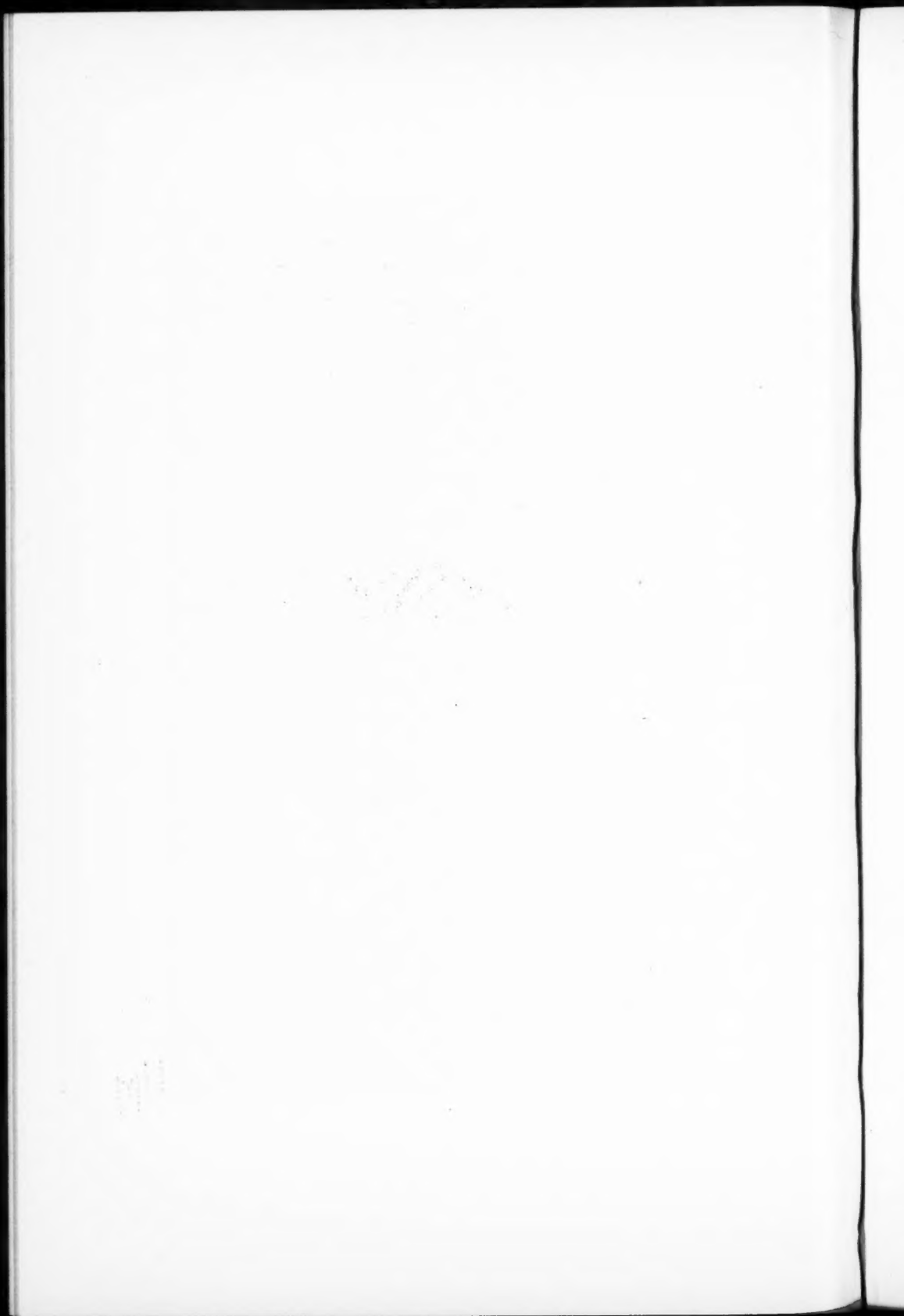
PLATE 3
PEAS FROM THE HORTICULTURAL PLOT AT MADISON, SHOWING ROOTS, INOCULATED AND
UNINOCULATED

FIELD TESTS ON THE INOCULATION OF CANNING PEAS

PLATE 3

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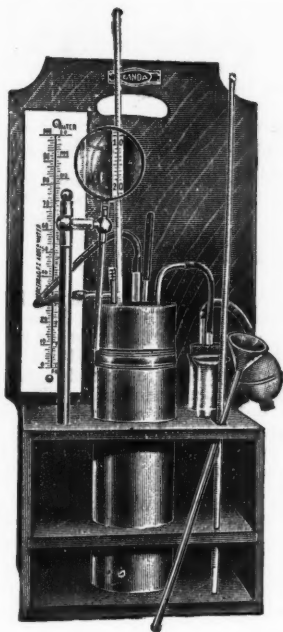
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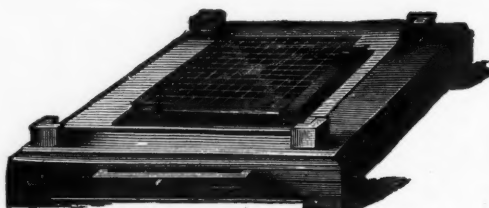
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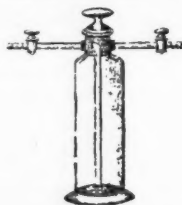
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